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A COMPARISON OF THE EFFECTS OF PHENOTHIAZINE DOSAGE WITH AND WITHOUT A MINERAL LICK SUPPLEMENT ON INDIGENOUS CALVES IN UGANDA

By

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INTRODUCTION

Under tropical conditons young cattle are frequently in poor condition. This is the result of a combination of factors of which seasonal variations in the food supply, restricted milk for calves, close grazing when herbage growth is short and parasitic infestation may be mentioned.

Investigations of worm egg counts in faeces and *post mortem* examinations of calves and goats at this Centre have established the presence of a variable nematode infestation. Worm egg counts are frequently high and a number of deaths directly attributable to parasitic gastro-enteritis occur. In such circumstances when the resistance of a calf is lowered by helminth infestation, its ability to withstand an infection of East Coast Fever is also reduced.

Previous work at this Centre (Randall, 1948) showed that a single dose of 20g. of phenothiazine produced adverse effects on the growth rate of calves. In fact, the average daily gain of the dosed calves was about 75 per cent of the gain of the untreated calves. This result suggested that further trials were necessary before the use of phenothiazine could be accepted as a means of controlling parasitic gastro-enteritis.

It was decided, therefore, to compare the effects of dosing at monthly intervals with phenothiazine with the effects of a mineral lick supplement, on the growth rate of calves at the Centre. At the close of the experiment the haemoglobin values, packed cell volume and inorganic phosphorus content of the blood were also examined.

MATERIALS AND METHODS

Materials

Cattle. Twenty-eight calves and young stock were available for the experiment. They were divided into four groups of seven animals each, as follows :—

Group (1), no treatment; group (2), phenothiazine alone once per month; group (3), phenothiazine once per month plus mineral lick *ad lib;* group (4), mineral lick alone.

All the animals had lived on the station either from birth or for at least a year prior to experiment. Many came originally from Teso

district in the Eastern Province. In each group one suckling calf was included. These were weaned after ten weeks, the experiment being continued for 34 weeks.

Phenothiazine dosage. Phenothiazine wettable powder was given as a drench in full dosage to the animals comprising groups 2 and 3. Dosage varied from 25 to 50 g. in one dose at monthly intervals. Animals weighing 150 lb. or under received 25 g., thereafter dosage was increased by 5 g. per 50 lb. body-weight up to 50 g. dosage for animals of 400 lb. Animals were dosed in the late afternoon and were housed overnight in order to lessen the risk of response to photosensitisation.

Mineral lick. The mineral licks available were compressed blocks weighing approximately 2,000 g. marketed by Messrs. Boots under the trade name of 'Mindif Mineral Licks.' These are stated to contain the following :—

Calcium, 3·4 per cent; Phosphorus, 0·65 per cent; Ca. to P. ratio, 5·2 to 1; Copper, 3000 ppm; Cobalt, 300 ppm; Manganese, 170 ppm; Iodine, 850 ppm; Iron, 0·35 per cent; Sodium Chloride, approximately 20 per cent.

Paddocks. Five small paddocks were available. These consisted principally of rough grazing of a poor quality. One group was allowed to each paddock each week and the groups were rotated weekly through the paddocks in order to ensure as uniform an environment as possible. Owing to the small size of these paddocks it became necessary to supplement the food of the animals and shortly after the commencement of the experiment each animal in each group was allowed 1 lb. of chopped cassava and ½ lb. of cotton seed per head per day.

Methods

Sampling. Groups were randomised on the basis of previous monthly weighings to give group weights of a similar total and therefore a similar average weight. Owing to an unfortunate clerical error in the weight of one animal a difference of 100 lb. is evident in Group 2, which was not discovered until some time after the experiment had commenced. This, therefore, has largely disrupted the method of randomisation.

Weighing. All animals were weighed weekly on an Avery cattle weigh-bridge which was checked each week before the weighing commenced.

Mineral lick supplement. The mineral licks were weighed before presentation to the cattle. They were laid in low concrete troughs approximately two feet square and were available to the cattle during the daytime but the licks were covered at night to minimise the effects of rain. After removal from the paddocks the licks were sun-dried and weighed again in order to furnish evidence of the intake of the lick.

Faeces samples. Samples were collected weekly from the rectum of each animal and counts were performed using a modification of the method devised by Whitlock and Gordon (1939) using 2 g. samples and 58 cc. of flotation fluid and using a two cell McMaster counting chamber, each chamber being filled with 0·15 cc. of mixture. Each count was duplicated and the mean figure of the counts of four chambers was used. Whenever there was a discrepancy of more than 100 eggs per gram a complete re-count was taken and the mean reading of 8 chambers was accepted for the egg count of that animal.

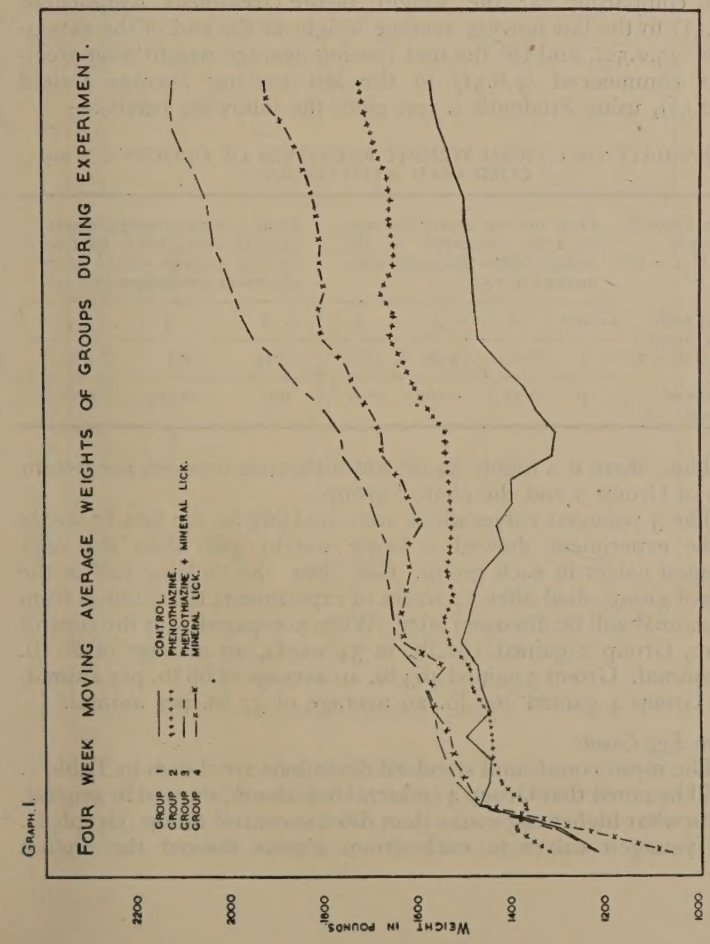
No attempt was made to identify the worm species by examination of larvae.

Biochemical examination. After twenty-six weeks on experiment, blood samples were collected for inorganic phosphorus estimation, three samples being collected during one week from each animal. They were examined by the method of Fiske and Subbarow (1925). The cell volume and haemoglobin contents were examined in the thirtieth week of the experiment and the packed cell volume was estimated using Wintrobe tubes. The haemoglobin was estimated in a Sahli Haemoglobinometer.

Daily rainfall. Measurements which are normally collected on the station were available for comparison during the experiment. (See Table 2.)

RESULTS

Our results indicate that while the monthly treatment with phenothiazine reduced the worm egg count considerably, the growth


GRAPH. I.
FOUR WEEK MOVING AVERAGE WEIGHTS OF GROUPS DURING EXPERIMENT.
GROUP 1 CONTROL.
GROUP 2 PHENOTHIAZINE.
GROUP 3 PHENOTHIAZINE + MINERAL LICK.
GROUP 4 MINERAL LICK.
WEIGHT IN POUNDS.
2200
2000
1800
1600
1400
1200
1000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
TIME IN WEEKS.

rate of the calves was not significantly different from that of untreated calves. There appeared to be a beneficial effect due solely to the addition of the mineral lick supplement and there was a highly significant increase in bodyweight of the group to which the joint treatment with phenothiazine and mineral licks was given.

Comparison of the Growth Rate as Judged by the Bodyweight of the Groups

It very soon became apparent that Group 3 which was receiving both the mineral lick and the monthly dosing of phenothiazine was in the best condition and showing the best weight gains. In the slight fluctuation of the weights which are experienced with weekly weighings of cattle the results have been compared using a four-week moving average to show the growth rate. These are compared in Graph I. Statistical examination of the weight relatives comparing (a) the weight before treatment commenced (9.7.51) to the last moving average weight at the end of the experiment (25.2.52), and (b) the first moving average weight after treatment commenced (7.8.51) to the last moving average weight (25.2.52), using Student's t. test gives the following result :—

PROBABILITY OF t. FROM WEIGHT RELATIVES OF GROUPS 2, 3 and 4 COMPARED WITH GROUP 1

No. in Control Sample $=n_1 + 1 = 6$	Final moving average weight (25.2.52) compared to the weight before treatment commenced (9.7.51)			Final moving average weight (25.2.52) compared to the first moving average weight after treatment commenced (7.8.51)		
No. in each tested sample $=n_2 + 1 = 7$	Group	2	3	4	2	3	4
	t	1·2	2·06	1·89	0·14	2·5	1·8
Degrees of freedom : 11	p	0·25	0·065	0·09	0·9	0·025	0·11

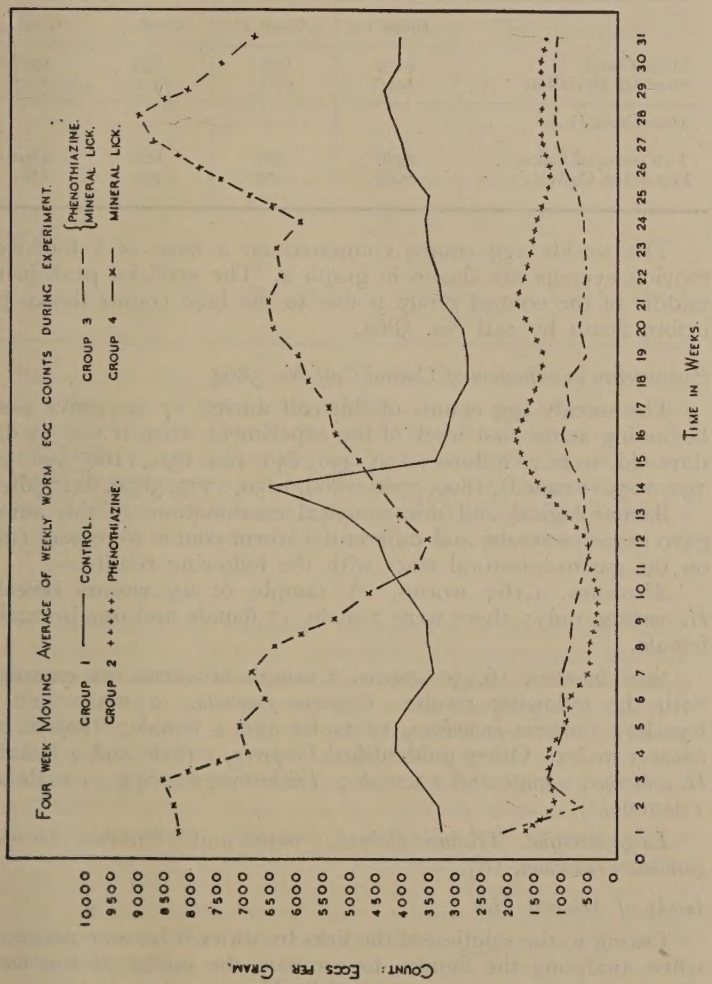
Thus, there is a highly significant difference between the weight gain of Group 3 and the control group.

The 3 youngest calves which were suckling for the first 17 weeks of the experiment showed a larger weight gain than the next youngest calves in each group. Calf 5864, the suckling calf of the control group, died after 17 weeks of experiment; the findings from this animal will be discussed later. When compared with the control group, Group 2 gained 182 lb. in 34 weeks, an average of 26 lb. per animal. Group 3 gained 463 lb. an average of 66 lb. per animal, and Group 4 gained 262 lb. an average of 37 lb. per animal.

Worm Egg Counts

The mean counts and standard deviations are shown in Table 1. It will be noted that Group 4 (mineral lick alone), showed in general, a somewhat higher egg count than did the control group (Graph 2). The youngest calves in each group always showed the highest

egg counts and it was frequently the counts in the youngest calves that were least affected by phenothiazine dosage. The post-mortem examination of calf No. 5864 showed that the predominant infestation was with *Cooperia* species.


Graph. 2.
Four week Moving Average of weekly worm egg counts during experiment.
Group 1 ——— Control.
Group 2 +++++ Phenothiazine.
Group 3 — - — {Phenothiazine. Mineral lick.
Group 4 — x — Mineral lick.
Count: Eggs per Gram.
10000 9500 9000 8500 8000 7500 7000 6500 6000 5500 5000 4500 4000 3500 3000 2500 2000 1500 1000 500 0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Time in Weeks.

Lütz (1948) reported this species as causing trouble in the Belgian Congo, while Andrews, Muniz and Ovihuila (1943) found that egg production was not markedly reduced in this species after phenothiazine treatment. Roberts (1951) noted that *Cooperia*

infestation in Queensland showed an infestation peak at an earlier age than with other species, usually at 4 to 5 months.

TABLE 1
SUMMARY OF RESULTS OF EGG COUNT

	Group 1	*Group* 2	*Group* 3	*Group* 4
Mean Count ..	4019	1369	893	6508
Standard Deviation ..	124·7	97·4	74·45	143·5
Mean Counts by Age				
Two youngest Calves ..	2486	381	660	4691
Five oldest Calves ..	1503	977	232	1781

The weekly egg counts compared on a basis of a four-week moving average are shown in graph 2. The artificial peak in the middle of the control group is due to the high counts shown just before death by calf No. 5864.

Post-mortem examination of Control Calf No. 5864

The weekly egg counts of this calf during 17 successive weeks beginning at the first week of the experiment when it was 53 days days old, were as follows : 150, 350, 250, 100, 650, 1100, 950, 500, 750, 1050 (weaned), 1800, 1700, 1500, 1250, 1775, 3650, 6225 (died).

Bacteriological and microscopical examinations of this animal gave negative results and differential worm counts were performed on the gastro-intestinal tract with the following results :—

Abomasum. 1,163 worms. A sample of 25 worms revealed *H. contortus* only; there were 7 male, 17 female and one immature female.

Small intestine. 16,450 worms. A sample of worms was examined with the following results : *Cooperia punctata,* 12 males and 23 females; *Cooperia oncophora,* 12 males and 1 female; *Cooperia pectinata* 3 males; Other unidentified *Cooperia,* 1 male and 4 females; *H. contortus,* 1 male and 1 female; *Trichostrongylus* Spp., 1 male and 1 female.

Large intestine. Trichuris globosa, 3 males and 8 females; *Oesophagostomum radiatum,* 107.

Intake of Mineral Lick

Owing to the solution of the licks by water it became necessary, when analysing the figures, to compare the intake during weeks when varying quantities of rain fell. The complete results are set out in Table 2. The intake, when calculated in grams per animal per day, showed a steady rise with the increase in rainfall. It also became evident that the animals of Group 3 showed a consistently larger intake of mineral lick than did those of Group 4. Disregard-

TABLE 2

AVERAGE OF MEAN DAILY INTAKE OF MINERAL LICK PER ANIMAL PER DAY

	Average Rainfall in Weeks							
	None	*One fall*	*0·1 in.*	*1·1 to 2·0 in.*	*2·1 to 3·0 in.*	*3·0 or more*	*All in average*	*Standard deviation*
Group 3 Phenothiazine and Mineral lick	16·46	19·36	20·50	18·62	25·24	29·17	21·27	11·955
Group 4 Mineral lick alone	10·74	11·97	13·00	15·86	20·65	22·58	15·48	8·567
Difference Group 3 minus Group 4	5·72	7·39	7·50	2·76	4·59	6·59	5·79	
X^2 P	42·32 <.01							

ing, however, the figures for weeks in which rainfall occurred and assuming the error to be due to solution of the lick, there was left the results for five weeks in which no rain fell. Thus the mean intake of mineral lick per animal per day in Group 3 was 16·46 g. compared with 10·75 g. per animal per day in Group 4. Mathematical treatment of the figures for these two groups in the five weeks showed a highly significant difference, ($ki^2 = 42·32$; P. = less than ·01).

Biochemical Investigations.

With regard to the inorganic phosphorus levels of the blood, which are shown in Table 3, there was little difference between the groups. As the standard deviation was small, the mean values for each group may be compared and when the groups are placed in order of the mean inorganic phosphorus level, an interesting result is obtained. The group showing the highest mean at each examination was Group 4 (mineral lick alone). Group 3 (mineral lick and phenothiazine) came next in order on two occasions, and Group 1 (no treatment) followed in order on two occasions, these groups reversing the order at the remaining examination. The group showing the lowest mean inorganic phosphorus level was Group 2, (phenothiazine alone) this position remaining constant at all three examinations. The results may be compared with those of Anderson (1933).

Cell Volume and Haemoglobin Value in the Various Groups

These results are summarised in Table 4. When compared with the Control Group there was a significant difference in the haemoglobin content of Group 2, but no significant difference in the

TABLE 3
MEAN INORGANIC PHOSPHORUS LEVEL IN BLOOD AND STANDARD DEVIATION

Group	1		2		3		4	
Date	*Inorg P*	*S.D.*	*Inorg P*	*S.D.*	*Inorg P*	*S.D.*	*Inorg P*	*S.D.*
21.1.52	4·98	0·074	3·57	0·64	5·04	0·048	5·05	0·063
24.1.52	5·17	0·305	5·10	0·044	5·26	0·039	5·78	0·081
26.1.52	5·80	0·054	5·36	0·047	5·26	0·039	5·83	0·062

cell volume. Group 4 showed a significant difference compared with the control group in both haemoglobin and packed cell volume. There are numerous records of changes in the haemoglobin and red blood cell count in non-ruminants, mostly occurring after prolonged heavy dosage of phenothiazine (Schnitzer, Siebenmann and Bett, 1942; Collier and Mack, 1944; Meriweather, 1941; Toynton, 1941). Holman and Pattison (1941, 1942), however, could not detect blood changes in sheep to phenothiazine dosage.

DISCUSSION

The properties and uses of phenothiazine have been reviewed by Davey and Innes (1942) and Edwards (1947); whilst its usefulness as an anthelmintic has been confirmed by many workers, (Taylor and Sanderson, 1940; Swales, 1943). Leiper and Peters (1941) concluded that the rate of growth was a reliable indication of the efficiency of an anthelmintic, but this is only correct when the nutrition of the animal is optimal. Phenothiazine mixed with salt has frequently been used (Habermann and Shorb, 1942) but reports of its use in conjunction with trace elements are exceedingly scanty and seem to be confined to sheep (Stewart and Crofton, 1943; Thorning, Sampson and Graham, 1944) and to horses (Dimock, 1949). Only Stewart and Crofton made a comparison of the effects and they found a significant additive effect between mineral supplement and phenothiazine treatment when using sheep grazing land unsuspected of mineral deficiency.

Garner (1950) has reported on mineral deficiency in cattle in West Africa. The present experiment tends to confirm work by Randall; for, where minerals are deficient, phenothiazine alone, while reducing the parasitic infestation does not, in fact, lead to an enhanced growth rate following the elimination of worms. We found, however, that when calves had access to a mixed mineral supplement their progress was better than when dosed monthly with phenothiazine in spite of a heavy worm infestation as judged by the faeces egg count under comparable conditions. This yields

TABLE 4
CELL VOLUME AND HAEMOGLOBIN VALUE

Group	*Animal No.*	*Age (Days)*	*Cell Vol.*	*Haemoglobin % Sahli*
Group 1 ..	342	803	25	74
	412	598	35	88
	436	491	33	76
	445	462	31	80
	5848	281	30	70
	5855	173	28	76
			Average 30·33	Average 77·33
			S.D. = 3·288	S.D. = 5·558
Group 2 ..	336	812	33	86
	5766	509	38	90
	440	480	29·5	74
	457	408	28	60
	5770	321	30·5	76
	5850	221	34 5	79
	5826	83	26	62
			Average 30·86	Average 75·85
			S.D. = 1·726	S.D. = 4.703
			X^2= 6·9776	X^2= 13·0131
			P=> ·30 not Significant	P=< ·05 Significant
Group 3 ..	344	799	31	75
	386	679	31	78
	441	478	33	75
	442	476	31	75
	468	374	28	66
	5849	264	33	80
	5863	83	29	68
			Average 30·86	Average 73·85
			S.D. = 1·726	S.D. = 4·703
			X_2 = 2·7658	X_2= 5·7377
			P=< ·70 Not Significant	P=< ·30 Not Significant
Group 4 ..	394	655	24	60
	5765	532	31	70
	446	461	27	68
	509	212	25	62
	5856	173	27	62
	5858	124	27	66
	5859	112	23	56
			Average 26·28	Average 64·33
			S.D. = 2·392	S.D. = 4.718
			X_2 = 11·1774	X_2 = 29·4343
			P=< ·05 Significant	P=< ·01 Highly Significant

presumptive evidence of mineral deficiency. Further, when the treatment with phenothiazine is combined with the mineral lick, the difference in growth rate between this group and the control, is then highly significant. This finding is in accord with that of Stewart and Crofton (1943), who found a similar effect. Again, the animals in Group 4 of the present experiment were able to gain more in weight than those dosed with phenothiazine, in spite of a heavy worm burden. This resistance to parasitic worms may be a factor of great significance in the tropics where parasitic infestation is widespread and calves are frequently in contact with adult stock. If a mineral supplement increases an animal's resistance to

intestinal parasites, it may well do so to other tropical diseases as for example, East Coast Fever. For many years African cattle owners have endeavoured to provide mineral supplements by taking their cattle to earth licks or in some cases providing them with rock salt.

There is much evidence in the literature of the effect of nutrition on parasitic infestation but no clear conclusion can be drawn. Roberts, O'Sullivan and Pick (1951) stated, "When nutritional conditions were good, cattle thrived while showing high egg counts, but when food deteriorated, cattle with similar egg counts showed severe clinical helminthiasis."

It has been found by Laurence, Groenewald, Quinn, Clark, Ortlepp and Bosman (1951) that worm infested sheep ate less hay while Filmer and McClure (1951) have demonstrated anti-nematode anti-bodies in the colostrum of the parasitised ewe and assert that they confer some protection on the suckling lamb. As resistance develops with age, any factors that help or enhance this resistance from the point of view of nutrition are to be encouraged. Thus it has been reported that lambs become infested at three to four months of age, and that symptoms follow in three to four weeks. A peak in the egg count is rapidly followed by a decline (Hawkins, Cole, Kline and Drudge, 1944). Stoll (1936) reported that one month old calves exposed to infection had negative faeces egg counts, later the calves showed a heavier egg count than adults; the highest counts being shown by animals seven to ten months of age. In the present experiment suckling calves showed a high faeces egg count and this would indicate that weaning is a particularly critical stage for the calf under conditions of heavy parasitic infestation.

In view of the apparent adverse effects previously found here after dosing with phenothiazine and the increased consumption of mineral lick by the treated Group, coupled with the additive effective of phenothiazine and mineral treatment, it would appear that phenothiazine alters in some way the mineral metabolism of the animal. It may well be that, where calves are receiving only a sub-optimal or even a deficient mineral intake, phenothiazine tends to convert a sub-clinical condition into a clinical disease. This view, which is strengthened by the fall in the inorganic phosphorus figures when the results are placed in order of groups (Table 3), might also explain some of the contradictory results in haemoglobin estimations following phenothiazine treatment. It is also in line with the remarks of Stewart (1945), "The most plausible explanation of toxic effects resulting from usually safe therapeutic dosages of phenothiazine is an alteration of the normally low level of absorption and prompt excretion of the drug due possibly to some abnormality of the intestinal tract, or more probably, in view of the incidence and distribution of the cases, to a dietary factor or factors."

The present experiment indicates that this factor may be the mineral metabolism of the animal.

SUMMARY

An experiment is described comparing the effects of phenothiazine alone, phenothiazine plus mineral lick and mineral lick alone on the body-weight increase in growing calves.

A marked reduction in the worm egg count was produced by the phenothiazine.

In spite of the reduction in egg count there was no significant difference between the body-weight increase of the control group and that in the group receiving phenothiazine alone.

An improved increase in body-weight, which was not statistically significant, was shown by the group receiving only a mineral lick supplement, in spite of heavy parasitic infestation.

A highly significant increase in bodyweight gain was shown by calves receiving both phenothiazine and mineral lick.

One calf in the Control Group died and differential worm count showed a predominance of *Cooperia* species.

The intake of the mineral lick was calculated and it was found that the larger amount taken by the group dosed with phenothiazine as compared with that taken by the group not so dosed was statistically significant.

At the termination of the experiment, the blood inorganic phosphorus level and haemoglobin and cell volume were estimated and the differences discussed.

ACKNOWLEDGMENTS

The writer is indebted to Mr. R. M. Bredon for the inorganic phosphorus estimation, to Mr. George Curry of the East African Statistical Department for help with mathematical calculations and to Mr. S. G. Laws for his work with haemoglobin and packed cell volume. Acknowledgment is due to the Chief Research Officer for his comments and to the Director of Veterinary Services and Animal Industry for permission to publish this paper.

REFERENCES

Anderson, A. W. (1933). *Imp. Bur. anim. Nut., Tech. Comm., No.* 4.

Andrews, J. S., Muniz, C. M., and Ovihuila, F. (1943). *J. Agric. Univ. Puerto Rico* **27,** 125. Quoted by J. T. Edwards (1947). *Imp. Agric. Bur. Joint. Publ. No.* 12.

Collier, H. B., and Mack, G. E. (1944). *Canad. J. Res. Sect. E.*, **22**, 1. (Abstr. in *Vet. Bull.*, 1944, **14,** 432).

Davey, D. G., and Innes, J. R. M. (1942). *Vet. Bull.*, **12**, R7.

Dimock, W. W. (1949). *Vet. Med.*, **44**, 99.

Edwards, J. T. (1947). "*Phenothiazine in Veterinary Medicine.*" *Imp. Agric. Bur. Joint Publ. No.* 12.

Filmer, D. B., and McClure, T. J. (1951). *Nature Lond.*, **168**, 170.

Fiske, C. H., and Subbarow, Y. J. (1925). *J. biol. Chem.*, **66**, 375.

Garner, R. J. (1950). *J. comp. Path.*, **60**, 108.
Habermann, R. T., and Shorb, D. A. (1942). *N. amer. Vet.*, **23**, 318.
Hawkins, P. A., Cole, C. L., Kline, E. E., and Drudge, J. H. (1944). *Vet. Med.*, **39**, 154.
Holman, H. H., and Pattison, I. H. (1941). *Vet. Rec.*, **53**, 491; (1942). *Ibid.*, **54**, 215.
Laurence G. B., Groenewald, J. W., Quinn, J. I., Clark, R., Ortlepp, R. I. and Bosman, S. W. (1951). *Onderstepoort. J. vet. Res.*, **25**, 1, 121.
Leiper, J. W. G., and Peters, B. C. (1941). *J. Helminth.*, **19**, 1971. (Abstr. in *Vet. Bull.*, (1942). **12**, 471).
Lütz (1948). *Rev. Elev. Med. vet. Pays trop.*, **2**, 165. (Abstr. in *Vet. Bull.* (1951). **21**, 120).
Meriweather, B. (1941). *Vet. Med.*, **36**, 374.
Randall, J. B. (1948). *Personal communication.*
Roberts, F. H. S. (1951). *Aust. vet. J.*, **27**, 274.
Roberts, F. H. S., O'Sullivan, P. J., and Pick, R. F. (1951). *Ibid*, **27**, 16.
Schnitzer, R. J., Siebenmann, C., and Bett, H. D. (1942) *Canad. publ. Hlth. J.*, **33**, 17. (Abstr. in *Vet. Bull.* (1942). **12**, 467.
Stewart, M. A. (1945). *J. Amer. vet. med. Assoc.*, **106**, 217.
Stewart, W., and Crofton, H. D. (1941). *Vet. Rec.*, **51**, 619; (1943). *Ibid.*, **53**, 3.
Stoll, N. R. (1936). *J. Parasitol.*, **22**, 386.
Swales, W. E. (1943). *Canad. J. comp. Med.*, **7**, 280. (Abstr. in *Vet. Bull.* (1944). **14**, 286).
Taylor, E. L. (1944). *Vet. Rec.*, **56**, 91.
Taylor, E. L., and Sanderson, M. (1940). *Ibid.*, **52**, 635.
Thorning, W. M., Sampsom, J., and Graham, R. (1944). *J. Amer. vet. med. Assoc.*, **104**, 67.
Toynton, C. (1941). *Vet. Med.*, **36**, 481.
Whitlock, H. V., and Gordon, H. McL. (1939). *J. Coun. sci. indust. Res. Austral.*, **12**, 50.

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A STANDARDIZED RAPID AGGLUTINATION TEST FOR THE QUANTITATIVE ESTIMATION OF *BRUCELLA* ANTIBODY IN MILK

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INTRODUCTION

In recent years the *Brucella abortus* ring test or abortus Bang ring test (A.B.R. test) has been extensively applied to brucellosis control in Europe and the U.S.A. It originated from the observation that certain minute particles, as of carbon, when added to raw whole milk, adhere to fat globules and rise with them to the surface, so giving a coloured surface band in the cream layer. It was later shown that a similar result could be obtained if coloured *Br. abortus* organisms were added, provided the milk sample contained specific antibody.

The A.B.R. test, though very useful when applied as a herd test, has not been adequately standardized and, in many ways, has not risen far above the empirical level; many authors, including Roepke (1950), have referred to its errors and inaccuracies. Bruhn (1948) who reviewed the literature on the test, demonstrated variability in sensitivity of antigens and stressed the occurrence of false positive reactions with unsuitable antigens. Although great accuracy cannot usually be expected in rapid tests, it is most undesirable that there should be wide divergences in results. With tests on bulk or herd milk, in which the bacterial content may be high, antigens stained with haematoxylin which were used in the earlier A.B.R. tests were likely to become decolourized by reduction. Substitution of tetrazolium salts has removed this disability.

During the last four years, in experiments on strain 19 vaccination (Gregory 1951, 1952) we have been able to examine quantitatively the antibody content of serum and milk obtained from several hundred vaccinated and unvaccinated animals exposed to natural infection as well as from many artifically infected cattle. With milk samples, an opportunity has been provided to devise a test which eliminates undesirable factors affecting the A.B.R. test, such as variation in the cream content of samples and the use of non-standardized antigens. Any satisfactory test on milk or whey, whether it be a ring test or an agglutination test, should provide a means of measuring the quantity of *Br. abortus* antibody therein. Montgomerie and Rowlands (1932) adequately reviewed the literature relating to the quantity of *Br. abortus* antibody in serum and in

milk. The discrepancies they found between agglutinin titres in whey and serum were quite comparable with differences recently described between the results of ring tests and serum agglutination tests. It is of interest to note that they mentioned that M'Fadyean and Stockman (1909) considered milk itself to be unsuitable for use in a diagnostic test on account of its opacity.

As the ring test has obviously provided a useful direct method of testing an opaque fluid for the presence of antibody, it was decided to examine the principles involved. It was considered that antibody should be quantitatively estimated and that the basis of a satisfactory antigen should be an adequately-coloured suspension of *Br. abortus* in the smooth state. The various methods of preparing stained antigens including the use of a mordant and the addition of glycerol, as well as the role of cream in the test, seemed to require special examination.

MATERIALS

Pointed glass tubes about 8 cm. in length with an average internal diameter of 7.7 mm. were found to be most suitable for testing 1 ml. quantities; the height of the column of fluid in the final test, as measured from the point of the tube to the meniscus of the fluid, will generally be in the range of 2.5 cm. to 3.0 cm. in batches of tubes with the usual variation in size.

Standard dropping pipettes (Donald, 1915) are prepared, to deliver 26 drops of water per ml. Ordinary calibrated glass pipettes are also required.

For the easy removal of relatively fat-free milk from beneath the layer of gravity cream formed in tubes or bottles containing milk samples, an automatic syringe, carrying a blunted 18 gauge needle three inches in length, is very useful.

Physical conditions found to be suitable for rapidity of the reaction require the use of rather narrow tubes and incubation in a water bath at 56°C; such requirements are similar to those used in some A.B.R. tests. The sets of tubes so prepared are placed in wire racks in a water bath at 56°C. so that about one-third of the column of fluid is immersed; this procedure ensures continuous mixing through the agency of convection currents. After a period of one hour the racks are removed from the water bath to the bench and, after standing for 30 min. at room temperature, the test is read.

METHODS

Preparation of Diluting Fluid

It was found that in the A.B.R. test the cream served only as a convenient vehicle for the transport of agglutinated coloured organisms to an observable surface band. It was therefore decided to alter radically the conditions for testing by removing the cream. Thus, on removal of the transporting agent the coloured organisms

in contact with antibody could no longer rise against gravity but, of necessity, tended to gravitate to the bottom of the tube as in an ordinary agglutination test.

The A.B.R. test is usually performed on one sample of whole milk in a single tube. Occasionally, when the quantitative estimation of antibody has been attempted, non-reactive whole milk has been used as a diluent.

Our early experiments on milk samples from individual cows indicated that considerable importance would generally be attached to a positive result obtained only if the antibody in a milk sample had been diluted five-fold or more and that, in quantitative work, it would be desirable, before adding the coloured bacterial suspension, to keep the opacity and physical properties of samples approximately constant in a range of dilutions from 1 in 5, onward. Therefore, in order that the opacity seen in a 1 in 5 dilution should not be diminished by the process of diluting antibody in the remainder of the series, it was decided to use a diluting fluid of similar nature.

Preparation of Diluting Fluid.

To ensure a constant supply, a convenient and simple procedure is to obtain fresh milk from a cow selected because her serum and milk are entirely negative in tests for *Br. abortus* antibody and to allow it to stand in a refrigerator or cold room until most of the cream has risen to the surface; the cream may then be conveniently removed with a pipette attached to a vacuum pump or, alternatively, the milk may be siphoned off from beneath the cream layer. Each volume of skim milk is then diluted by the addition of four volumes of 0.85 per cent. sodium chloride solution and 0.5 per cent. of phenol is added as a preservative. A stock of phenolized non-reactive skim milk may also be kept for later dilution with phenol saline to 1 in 5, as required.

Preparation of Bacterial Suspension

Bacterial suspensions were prepared from strain 99 (Weybridge), which is used in England for routine serological work, and also from a culture of strain 19 currently used by us for the preparation of vaccine both of which proved suitable. Suspensions of these strains had also proved satisfactory for use in serum agglutination tests when performed by the Weybridge technique against an international standard agglutinating serum issued from Weybridge. They were maintained in a smooth state.

The principles involved in preparing a bacterial suspension in readiness for colouring are essentially those of the usual method of production of *Br. abortus* strain 19 vaccine or suspensions used in routine serum agglutination tests. In the work reported here, potato-extract agar has been used in flat bottles but incubation after sowing has been restricted to about 40 to 44 hours because

sufficient growth has then occurred and because more prolonged incubation increases the number of non-viable bacteria and, therefore, affects the intensity of colour later developed when a tetrazolium salt is added. The growth is washed off with approximately 15 ml. of pancreatic-digest broth (Brown, 1948) for every 20 square inches of surface growth and some of the suspension is sown on the surface of a plate of 2 per cent. glycerol 1 per cent. dextrose agar which is examined after 4 days incubation to confirm the smoothness of the culture.

Preparation of Coloured Antigen

The disadvantages of haematoxylin as a staining agent, when compared with reduced tetrazolium salts, soon became apparent and the use of tetrazolium was therefore adopted. The addition of glycerol did not significantly improve the test.

The importance of alum in the preparation of antigen required more consideration. It may well serve as a mordant in haematoxylin staining but its usefulness in tests involving agglutination may not be restricted to that action. Tervalent cations present in an agglutinating system may exercise a profound effect. Experiments were, therefore, conducted to determine whether alum leaches into the suspending fluid in sufficient quantity to influence the agglutination reaction in tests with diluted milk or with saline as diluent. Such an effect was found to be negligible, presumably because attachment to the organisms was firm. However, alum-treated suspensions, even after washing, often showed a tendency to agglutinate in the presence of physiological saline, in the absence of serum. This saline sensitivity is eliminated by the addition of about 1 per cent. of protective colloid in the form of milk or serum. In a final analysis of the experimental data, it was concluded that although alum treatment of suspensions might slightly increase the sensitivity of ring tests, its greater importance in the standardized agglutination test to be described is that it reduces or eliminates zoning which occurs in some samples of milk tested with untreated suspension. For this reason it was decided that it might be retained with advantage.

Pancreatic broth provides a suitable menstruum for bacterial respiration during the next stage when 0.1 per cent. of 2, 3, 5-triphenyl tetrazolium chloride is added immediately the suspension is prepared and the flask is then placed in an incubator at 37°C. for 2 to $2\frac{1}{2}$ hours with occasional shaking. Physiological saline is not used as suspending fluid because it retards bacterial respiration.

At the end of the incubation period the suspension should be of a deep dusky red colour. It is heated immediately in a water bath at 60°C. for one hour to kill the organisms; it may then be kept overnight in a refrigerator at about 4°C. before proceeding with further treatment. Finely-powdered ammonium alum is next added to give a

concentration of 7 per cent. and the suspension is held at 37°C. in a water bath or incubator for 2 hours with occasional shaking.

The suspending fluid containing dissolved alum is now removed from the bacterial deposit after centrifugation. The supernatant fluid is replaced by about three-fourths of its volume of 0.85 per cent. saline containing 0.5 per cent. of phenol and the bacteria are resuspended by adequate mixing. Finally, the suspension is shaken in a flask containing glass beads to break up clumps, as the bacteria tend to stick together after alum treatment.

Standardization of Antigen.

The bacterial content of the coloured suspension, prepared as described, is estimated by the Hopkins tube technique. Usually it is found to exceed 6 per cent.

Measured amounts of well-shaken suspension (e.g., about 1 ml.) are placed in five 2 ml. tubes and appropriate amounts of 0.5 per cent. phenol saline are added to give a range of suspensions with a bacterial content increasing by steps of 0.3 per cent. from 4.7 per cent. to 5.9 per cent.

For the next step a dried standard *Br. abortus* agglutinating serum as issued from the laboratories of the Ministry of Agriculture and Fisheries at Weybridge, England, is reconstituted and added to diluting fluid to give a dilution of 1 part of standard serum in 40. From this supply of the basic dilution a further set of dilutions is made, in which the dilution factors are 160, 320, 360, 400, 440, 480, 520 and 560. From each of these dilutions, 1 ml. is measured into five agglutination tubes to give five identical series of 8 tubes covering that range.

From each of the five graded dilutions of coloured bacterial suspension previously prepared, one drop is measured with the standard dropping pipette into all tubes of one row containing the range of dilutions of standard serum. As each row is finished the antigen and serum are well mixed in the tubes and incubated at 56°C. for 1 hour followed by 30 minutes at room temperature.

In this test it has been found convenient to describe degrees of agglutination as follows:—(1) less than 25 per cent. of the bacteria agglutinated as judged by the amount of sediment and a considerable amount of colour in the supernatant fluid: (2) about 25 to 50 per cent. of the bacteria agglutinated and a moderate amount of colour in the supernatant fluid: (3) the majority of the bacteria agglutinated and some colour left in the supernatant fluid: (4) complete agglutination and the supernatant fluid with the milky white colour of the diluting fluid.

The end-point of agglutination is taken as occurring in the last tube in the series which gives a reading of at least 2; it is a point where appreciable agglutination is quite evident. There should not be difficulty for any laboratory to fix an end-point, at about this level, which will enable results to be reproduced in repeated tests.

In this titration a suspension giving an end-point reading with the serum diluted 1 in 400 to 1 in 480 is satisfactory for use in the routine milk agglutination test but the most sensitive, within that range, is to be preferred. If the most concentrated bacterial suspension being tested were to agglutinate in a serum dilution above 1 in 480 it would be necessary to prepare a still more concentrated suspension in order to bring the end-point within the desired range. Any suspension more dilute than 4.7 per cent. is unsatisfactory as it does not give enough bulk of agglutinated bacteria for easy reading.

Having determined a dilution of bacterial suspension giving the desired end-point, the bulk sample of suspension is adjusted to that strength with 0.5 per cent. phenol saline and kept in the refrigerator for use in routine tests.

Technique of the Rapid Milk Agglutination (R.M.A.) Test

Samples of milk should be collected as fresh as possible in tubes or screw-cap bottles and forwarded to the laboratory in such a manner as to avoid any great change in the bacterial content. If milk samples cannot be kept cold during any lengthy period of transport to a laboratory, up to one per cent. of boric acid or of commercial formalin can be added without altering the results of the test, but, as a routine procedure, 0.1 per cent. of formalin is adequate.

At the laboratory, samples are allowed to stand undisturbed overnight and, if unpreserved, they should be placed in a refrigerator or cold room. Next day, or later, about 3 ml. of relatively fat-free milk is obtained; one ml. is transferred to a tube containing 4.0 ml. of saline, to give a basic 1 in 5 dilution, and the remainder is kept in reserve as undiluted milk.

In a complete quantitative test, a series of tubes is set up and one volume (1.0 ml.) of diluting fluid is pipetted into all except the first and second. To the first tube is then added one volume of the undiluted sample and to the second and third tubes one volume of 1 in 5 dilution. After mixing, the extra volume of 1 in 10 dilution in the third tube is now transferred to the fourth and a similar procedure is continued until the last tube is reached when a volume is discarded. Thus, in addition to one volume of undiluted milk in the first tube, there is, in the remainder, a series of doubling dilutions commencing at 1 in 5 and, because of the nature of the diluting fluid, all dilutions have the colour and gross physical properties of milk diluted 1 part with 4 parts of saline.

To all tubes, one drop of standardized antigen is then added from the dropping pipette which delivers 26 drops of aqueous fluid per ml. As the drop of antigen should not be allowed to settle at the bottom, mixing should take place after adding antigen to about 12 to 15 tubes. The end-point of the reaction after incubation is that described in the procedure for the standardization of antigen.

DISCUSSION

The rapid milk agglutination (R.M.A.) test, as described, provides a simple and practical method of estimating *Br. abortus* antibody in milk; moreover, it is a standardized procedure by means of which comparable results may be obtained in different laboratories. The standardization of the test depends upon the use of a standard serum, a procedure which has been recognized, internationally, as suitable for the standardization of serum agglutination tests when the aim is to maintain uniformity of results in routine diagnostic tests.

In the study of bovine brucellosis, much academic interest attaches to the antibody content of milk. Previously the whey agglutination method, which is comparatively slow and rather cumbersome, has been used in quantitative work. The results of the R.M.A. test, after standardization, approximate very closely to those obtained in whey agglutination by use of the Weybridge suspension and technique, as applied in serum tests; in the great majority of instances the results are identical. Similarly, if sera of known titre are diluted with 1 in 5 non-reactive milk instead of in saline and are submitted to the R.M.A. test the results will be practically identical with those of the Weybridge serum agglutination test. Occasional slight variations by one tube, or enough to cause doubt regarding identity of end-points, are to be expected in comparing the results of any rapid test with those of a lengthy tube agglutination test.

In this laboratory it has been possible to compare, in infected or vaccinated cattle, the antibody content of milk and serum by the Weybridge serum agglutination test and the R.M.A. test respectively, so that antibody in each fluid is measured in the same quantitative units. Although such hypothetical units differ from those in the B.A.I. test or other recognized standardized serum tests they can be converted from one to the other by the application of the conversion factor ascertainable from serum agglutination tests standardized by means of the international standard dried serum. The minimum diagnostic end-point of 1 in 40 in the Weybridge serum test, corresponds to an end-point of 1 in 100 in the B.A.I. test (Report, 1948).

In theory it should be possible to adjust the concentration and sensitivity of an R.M.A. antigen so that antibody could be measured directly in B.A.I. units but, in practice, there are then too few bacteria present in the test volume of fluid to give an end-point which is easy to read.

The R.M.A. test may be used instead of the *Brucella abortus* (A.B.R.) test for the examination of samples of milk from cans or larger bulk samples. When undiluted milk is used, a little extra care must be taken to avoid admixture with cream when the milk is

removed from beneath the layer of gravity cream; otherwise some of the coloured bacteria will be diverted to form a coloured ring at the top of the tube.

Like the A.B.R. test, the appearance of positive reactions in bulk milk depends upon the quantity of antibody secreted in the milk of individual infected cows and this varies according to the stage of lactation and the serum titre. Some individual animals secrete milk which, after being diluted 20,000 times, will give a positive reaction in this R.M.A. test or in a whey agglutination test, based on Weybridge standard procedures. A full discussion of the applications of the R.M.A. test as an aid to the diagnosis of brucellosis in individual cows and in herds will be published at a later date.

The R.M.A. test, which involves the use of an alum-treated antigen, eliminates many false negative results, due to zoning, which are recorded in whey agglutination tests.

SUMMARY

A rapid milk agglutination test for the detection of *Br. abortus* antibody in milk is described.

This test is easy to perform and it is standardized against standard dried serum recommended for use, internationally, in serum agglutination tests.

The test is of value in quantitatively estimating specific antibody in milk.

The test can be used as an aid to diagnosis of brucellosis not only in herds but also in individual cows.

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REFERENCES

Brown, J. H. (1948). *J. Bact.*, **55**, 871.
Bruhn, P. A. (1948). *Amer. J. vet. Res.*, **9**, 360.
Donald, R. (1915). *Lancet, ii*, 1243.
Gregory, T. S. (1951). *Aust. vet. J.*, **27**, 319; (1952). *Ibid.*, **28**, 194.
M'Fadyean, J., and Stockman, S. (1909). *Rept. Comm. Epizootic Abortion. Appendix Pt.* 1. London.
Montgomerie, R. F., and Rowlands, W. T. (1932). *Vet. J.*, **88**, 227.
———— (1948). *Chief B.A.I., Agric. Res. Admin., U.S. Dept. Agric.*
Roepke, M. H. (1950). "*Brucellosis*" (Symposium) p. 126. American Association for the Advancement of Science, Washington, D.C., U.S.A.

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LEUCOCYTE INVASION OF THE GENITAL TRACT OF AN INTERSEXUAL GOAT

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INTRODUCTION

It is well known that intersexes are common amongst domestic goats. These animals have been ranged in series by various investigators, from almost normal males through all degrees of intersexuality to almost normal females (Crew, 1923 a and b; Degode, 1940; Kondo, 1949; Eaton, 1943). The morphology and, to some extent, the histology of the genitalia have been used as criteria.

The intersexual condition appears to be inherited as a recessive character which is closely linked with the dominant gene for hornlessness (Hellyer, 1946; Asdell, 1946; Holmes, 1945; Ottaway and Arnold, 1945; Eaton, 1944). Opinion is divided upon the question as to whether these animals are genetically males or females (Crew, 1923 a and b, 1926 a and b; Somervail, 1937; Hill, 1941; Eaton, 1944; Asdell, 1944; Eaton and Simmons, 1939; Zimmermann, 1948; Folley, 1944; Crew and Dighton, 1923; Crew, 1922).

MATERIAL AND METHODS

This report is based upon the study of a kid which was the result of the first pregnancy of a two year old, crossbred, white, hornless nanny. Its sire was a three year old British Saanen hornless billy. No twin was reported at the time of birth of this animal, and we have no information to suggest that there was any twin foetus at an earlier stage of this pregnancy, when a freemartin condition might have been possible.

Several photographs of the animal were taken while it was still alive, including views of the external genitalia (Fig. 1). When the kid was three days old it was killed and photographed at successive stages of dissection (Fig. 2). The complete genital tract was removed and fixed in Bouin's Fluid (Fig. 3). Portions of the genitalia were selected and histological sections made at the positions indicated in Fig. 3. The tissues were embedded in paraffin wax and sections were stained with Ehrlich's haematoxylin and eosin, Masson's trichrome, and Gram's stain for observations bacteria.

OBSERVATIONS

External Genitalia

On the ventral surface of the abdomen, just caudal to a pair of wellmarked teats, scrotal swellings were visible and subcutaneous gonads could be palpated. From this aspect the under-surface of the

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short phallus was also visible. On elevation of the tail the anus was exposed, and also the urinary outlet at the base of the phallus on the side facing towards the anus. A typical vulval opening, bounded by vulval lips, was not present, but instead was found a short raphe extending from the urinary to the anal orifice (Fig. 1).

The phallus, which was intermediate in structure between a penis and a clitoris, was directed caudally and terminated in a glans-like enlargement, but no urethral canal was present within it. Its substance consisted of dense fibrous and erectile tissue covered with hairy skin proximally and thin hairless skin in the region of the glans.

Internal Genitalia

The animal was killed and dissected in the fresh condition. Removal of the skin from the ventral surface of the abdomen exposed the gonads and spermatic cord within the tunica vaginalis, and lying external to the musculature of the body wall. Within the sac of the tunica vaginalis were found the gonad and structures later identified as the head and tail of the epididymis (Figs. 2, 3 and 4). The tail of the epididymis was noticeably hyperaemic, while the head of the epididymis and the gonad were whitish in colour. In the spermatic cord were a vascular plexus and both Müllerian and Wolffian duct derivatives. The Müllerian and Wolffian ducts passed inwards and then dorsal to the bladder to merge in the tissue of the median genital cord. At this stage the genital tract was removed from the animal and fixed in Bouin's fluid before proceeding further with the dissection (Fig. 3).

Female components. The Müllerian duct (uterine tube) arose in the region of the hyperaemic tail of the epididymis and shared a common mesentery with the Wolffian duct (vas deferens) (Figs. 3 and 5). The uterine tubes passed into the abdominal cavity via the inguinal canal, becoming the uterine horns, and eventually fused with each other in the genital cord to form the body of the uterus. This uterine body passed, via a muscular cervical canal, into a laterally expanded vaginal segment which did not possess an opening caudally into the urogenital sinus but ended blindly as a solid epithelial cord. The body of the uterus and the vagina were situated in the dorsal half of the genital cord, that is in a position dorsal and medial to the male structures to be described later (Figs. 3, 6 and 7).

The uterine horns and the uterine body showed a histological structure characteristic of a normal, immature uterus. They consisted of a serosal covering, a muscularis and an endometrium of cellular connective tissue lined by a simple epithelium (Figs. 5 and 6). The primordia of the uterine glands extended at intervals from the epithelial lining into the underlying connective tissue.

The vagina was both long and wide, but dorso-ventrally compressed (Figs. 3 and 7). Its wall consisted of a fibrous adventitia, a thin muscularis and a mucosa of fairly fibrous vascular connective

tissue which was lined by an epithelium 3 to 5 cells thick. The lumen of the vagina gradually became obliterated at the caudal end, the epithelial lining ending as a solid cord of cells abutting against the anterior end of the dorsal wall of the urogenital sinus (vestibule). The urogenital sinus itself was a tubular structure extending from its contact with the solid caudal end of the vagina and entry of the urethra to the point at the base of the phallus where the urine was voided.

Male components. On examination of histological sections of the gonadal complex a testis and a head and tail of an epididymis were identified (Figs. 2, 3 and 4). The testis consisted of a fibrous capsule containing a mass of solid, immature testis tubules, with cellular connective tissue between them. A few of the interstitial cells were typical, polygonal, glandular cells.

The epididymis showed the usual subdivision into head and tail, but the macroscopic and microscopic appearance of the head was different from that of the tail. The head was pale in colour in the fresh state, and on histological sectioning was found to be composed of well-defined tubules lined by tall columnar epithelium and bound together with connective tissue. The tail, however, was divisible into a cortical region, structurally like the head, and a distinct medullary region. This central zone of the tail of the epididymis consisted of tubules with a very large lumen lined by a stratified cuboidal epithelium. Furthermore, the whole of the medullary tissue was infiltrated with myriads of polymorphonuclear leucocytes. These leucocytes appeared to have strongly acidophilic cytoplasm, but the refractile granules characteristic of eosinophilic polymorphonuclear leucocytes were not present. It is possible that the granules may not have been preserved during fixation as the whole genital tract was fixed in bulk in Bouin's fluid. The lumen of part of one of the central tubules was so distended with polymorphs that it formed a large "vesicle" (Fig. 4). A section of this region was stained with Gram's stain, but no bacteria were detected. This differentiation of the tail of the epididymis into two regions was bilateral, although the distribution of polymorphs was less sharply delimited on the left than on the right. From the region of the tail of the epididymis the vas deferens passed along the spermatic cord alongside the uterine tube to enter the tissues of the genital cord. In the region of the spermatic cord the vasa deferentia were smaller in diameter than the uterine horns, and were lined by a stratified epithelium which was intensely invaded by polymorphs. Although the derivatives of the Müllerian and Wolffian ducts were sharing a common mesentery the leucocytic invasion was restricted to the Wolffian duct component (Figs. 5 and 6).

On reaching the genital cord the vasa deferentia remained separate from one another, but increased in diameter and pursued their course caudally in a position ventro-lateral to the uterine body

(Figs. 3 and 6). The lumen of each vas was very irregular and the stratified epithelial lining hypertrophied. Numerous polymorphs were passing through the epithelium and packing the lumen (Figs. 6 and 10). Further caudally, at the level of the vaginal segment and ventral to it, the vasa deferentia were broadly attached to a pair of lobulated seminal vesicles (Figs. 3 and 7). The seminal vesicles were also lined by an irregularly hypertrophied, stratified epithelium and showed a similar intense invasion by polymorphs.

Beyond the level of the seminal vesicles the solid end of the vagina (dorsal), the paired vasa deferentia (middle), and urethra (ventral), bound together by connective tissue, continued caudally (Fig. 8). A short distance behind the vagina the ejaculatory ducts and urethra entered the urogenital sinus (Fig. 9). Leucocytic invasion of Wolffian derivatives was still a striking feature in the rest of their course, and indeed the openings of the ducts into the urogenital sinus are marked by "halos" of darkly-staining leucocytes in a section of this region (Fig. 9).

DISCUSSION

The most remarkable feature of the genital tract of the specimen described was the bilateral invasion of the whole male duct system by leucocytes, and the almost complete absence of these cells from the neighbouring female components. It is perhaps unwise to lay too much stress upon a feature reported in only one case, but it would be interesting to know if a similar infiltration occurs in other cases of intersexuality. If this were so, then the condition would appear increasingly significant.

Although the gonad is distinctly a testis, it is the male duct system that appears to be degenerating and is subjected to the intense leucocytic invasion, while the female ducts appear histologically quite healthy. The animal was killed three days after birth and no bacteria were detected in the sections stained by Gram's stain.

SUMMARY

A three day old intersexual goat was subjected to a complete morphological and histological examination of the genitalia. The external genitalia were intermediate in character between the female and male condition. The lateral lips of the urogenital sinus had fused to form a short raphe, but the urine reached the exterior at the proximal end of the phallus. The phallus consisted of erectile and fibrous tissue, but did not possess a urethral canal and could be called a rudimentary penis or enlarged clitoris. The specimen was found to possess, as female structures, Müllerian ducts forming uterine tubes, horns, body and cervix and a large vagina ending blindly against the vestibule. As male structures there were present

PLATE I

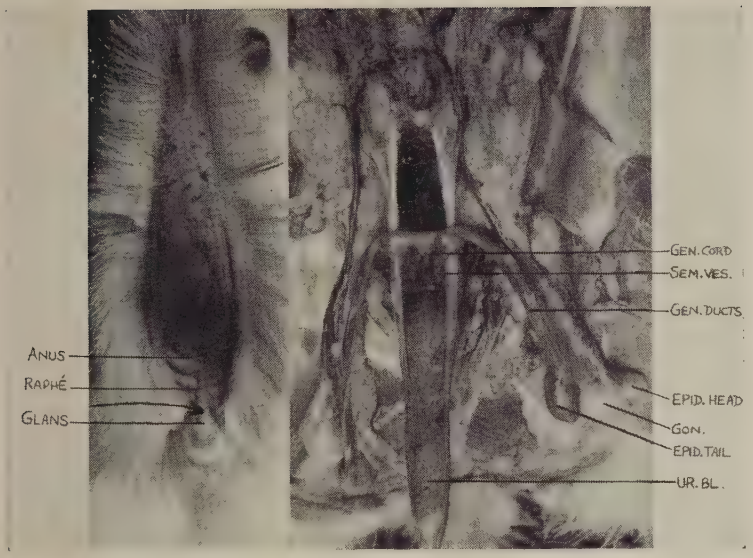


Fig. 1. Fig. 2.

FIG. 1. External genitalia of living intersexual goat. The tail is elevated to reveal the anus and phallus. The external opening of the urogenital sinus, the point at which urine reaches the exterior, is indicated by the arrow.

FIG. 2. View of the internal genitalia exposed during the course of dissection of the freshly killed animal. The skin has been removed from the ventral surface of the abdomen and the body wall musculature cut away. The parietal layer of the tunica vaginalis has been cut away to expose the gonadial complex and the bladder deflected to show the genital ducts entering the genital cord. The darker colour of the tail of the epididymis is due to its vascularity.

For abbreviations to figures, see page 183.

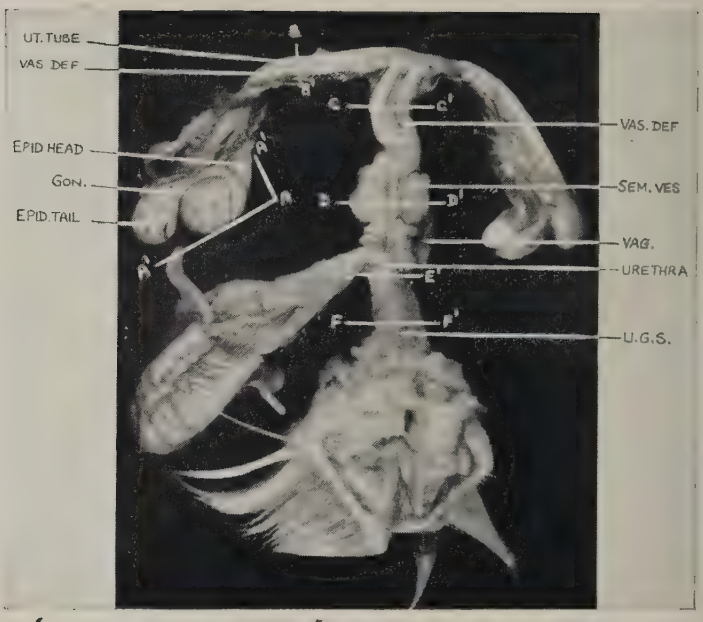


Fig. 3.

FIG. 3. View from the ventral aspect of the complete genital tract of intersexual goat after its fixation in Bouin's fluid and its removal from the animal. Lines A–A^1. B–B^1, etc., indicate the position at which histological sections were prepared, (See Plate III.)
× 0.6

PLATE III

Photomicrographs of histological sections of the genital tract of an intersexual goat. (For orientation of these sections see Plate II.)

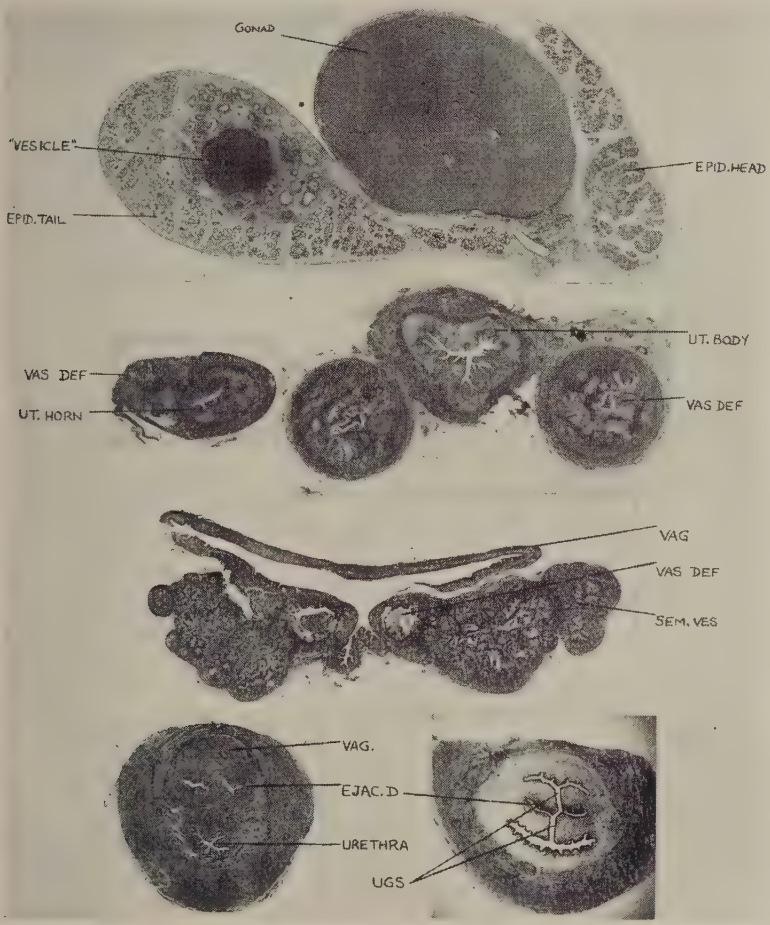


FIG. 4. Section A–A¹ passes through the gonadial complex of the right side. The tail of the epididymis shows a subdivision into two zones, the central one of which is composed of larger tubules invaded by and containing numerous leucocytes. The large, dark, central area of the inner zone of the tail of the epididymis consists of a vesicle of leucocytes darkly stained by haematoxylin. × 3.

FIG. 5. Section B–B¹ passes through the Mullerian and Wolffian duct derivatives in the region of the spermatic cord. The vas deferens lies near to the mesenteric attachment and is invaded by leucocytes. The uterine horn lies in the antimesometrial region and has the histological structure of a normal, immature uterine horn. × 5.

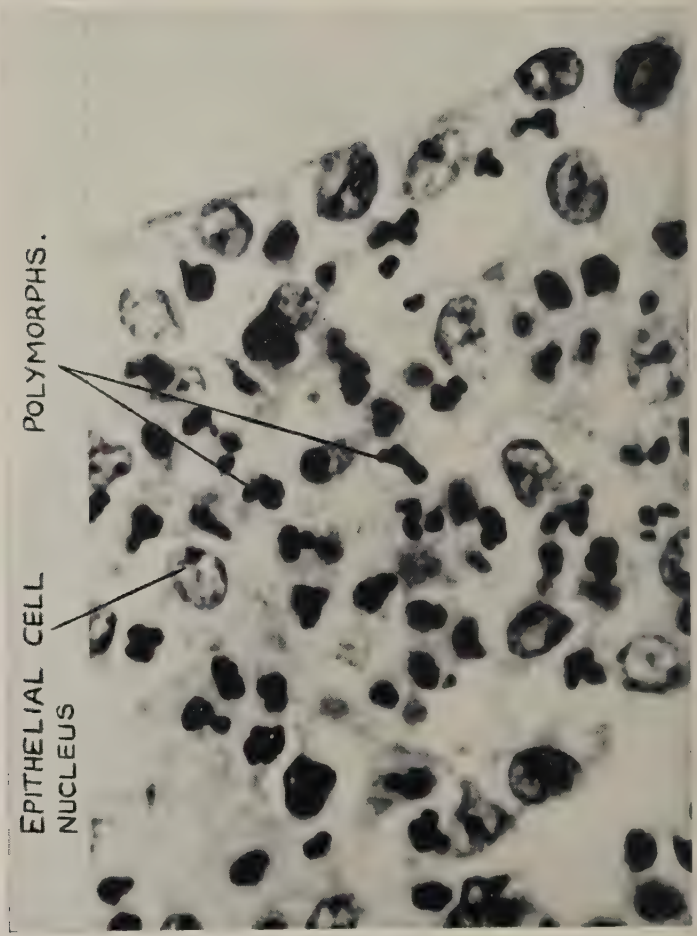
FIG. 6. Section C–C¹ passes through the anterior region of the genital cord, cutting the uterine body and the vasa deferentia. These have a very irregular lumen and are intensely invaded by leucocytes, while the uterine body appears histologically normal. × 7.

FIG. 7. Section D–D¹ passes through the vagina, vasa deferentia and seminal vesicles. The seminal vesicles and vasa are invaded by and contain numerous leucocytes. × 3.

FIG. 8. Section E–E¹ passes through the solid epithelial end of the vagina, vasa deferentia, ejaculatory ducts and the urethra. The leucocyte invasion still affects the Wolffian duct derivatives at this level. × 3.

FIG. 9. Section F–F¹ passes through the urogenital sinus in the region where the ejaculatory ducts are entering it, posterior to the solid end of the vagina and the entry of the urethra. The dark areas surrounding the points of entry of the ejaculatory ducts are due to the presence of numerous leucocytes. × 3.

PLATE IV



H.P. Photomicrograph of epithelium of Wolffian duct in region of uterine body (see Fig. 6), to show its invasion by polymorphonuclear leucocytes.

testes, epididymes, vasa deferentia, seminal vesicles and ejaculatory ducts opening into the urogenital sinus (vestibule). The whole Wolffian system was invaded by polymorphonuclear leucocytes with acidophil cytoplasm. No bacterial infection was detected. This feature of the case is briefly discussed.

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REFERENCES

Asdell, S. A. (1944). *Anim. Breed. Abstr.*, **12**, 88; (1946). *Ibid.*, **14**, 156.
Boring, A. M., and Kay, Y. (1936). *Ibid.*, **6**, 24.
Crew, F. A. E. (1922). *Vet. J.*, **78**, 133, 461; (1923a) *Ibid.*, **79**, 173; (1923b). *Proc. Roy. Soc.* (*B*), **95**, 90; (1926a). *Quart. Rev. Biol.* **1**, 315; (1926b). *Ibid.*, **2**, 427.
Crew, F. A. E., and Dighton, A. (1923). *Vet. J.*, **79**, 135.
Degode, F. (1940). *Anim. Breed. Abstr.*, **16**, 135.
Eaton, O. N. (1943). *Amer. J. vet. Res.*, **4**, 333; (1944). *Genetics*, **30**, 51.
Eaton, O. N., and Simmons, V. L. (1939). *J. Hered.*, **30**, 261.
Folley, S. J. (1944). *Anim. Breed. Abstr.*, **12**, 144.
Hellyer, G. C. (1946). *Ibid.*, **14**, 237.
Hill, R. T. (1941). *Ibid.*, **10**, 36.
Holmes, W. F. (1945). *Ibid.*, **13**, 94.
Kondo, K. (1949). *Ibid.*, **17**, 68.
Ottaway, C. W., and Arnold, R. M. (1945). *Vet. J.*, **101**, 150.
Somervail, M. L. (1937). *Anim. Breed. Abstr.*, **7**, 324.
Weber, W. (1944). *Ibid.*, **12**, 89.
Zimmermann, G. (1948). *Ibid.*, **16**, 291.

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ABBREVIATIONS TO FIGURES

Epid. head	Head of epididymis
Epid. tail	Tail of epididymis
Ejac. D	Ejaculatory duct
Epith. cell nuc.	Epithelial cell nucleus
Gen. cord	Genital cord
Gen. ducts	Genital ducts
Gon.	Gonad
Poly.	Polymorph
Sem. ves.	Seminal vesicles
U.G.S.	Urogenital sinus
Ur. bl.	Urinary bladder
Ut. body	Uterine body
Ut. horn	Uterine horn
Ut. tube	Uterine tube
Vag.	Vagina
Vas def.	Vas deferens

BIOCHEMICAL ASPECTS OF RUMINANT KETOSIS

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INTRODUCTION

Since Stinson (1929) drew attention to the fact that post-parturient dyspepsia of bovines was accompanied by the presence of ketone bodies in the milk and urine, there have been a number of biochemical investigations into the changes in that disease, which is frequently called acetonaemia or bovine ketosis. The same is true of pregnancy toxaemia or twin lamb disease of ewes. These observations have usually been limited, however, so far as ketone bodies are concerned, to the determination of the total amount present in the blood and urine and the results are quoted in Tables 1 and 2. These give data from normal cows and from cases of bovine ketosis respectively. In the few instances where there has been an attempt at partition, acetone and acetoacetic acid have usually been determined together and said to comprise 20 to 40 per cent. of the total in the blood of normal cows (Boddie, 1935; Knodt, Shaw and White, 1942) and from 23 to 80 per cent. in ketosis cases (Sampson, Gonzaga and Hayden, 1933; Boddie, 1935; Steyn, 1941); the remainder

TABLE I

TOTAL KETONES IN NORMAL COWS

(Mg. Acetone per 100 ml.)

Reference	*Material Examined*	*No. of Samples*	*Mean*	*Range*
Sampson *et al*, 1933	Blood	30	2·98	2.11–4·66
	Urine	9	7·83	3·45–13·15
Boddie, 1935	Blood	50	2·18	0·69–9·90
	Urine	16	3·27	2·50–6·37
Duncan, Huffmann and Tobin, 1939	Blood	176	2·99	0·66–5·54
	Urine	86	10·00	2·50–70·0
Sampson and Boley, 1941	Blood	8	1·92	0·87–3·97
	Milk	8	0·0	nil
	Urine	8	22·01	4·96–34·46
Knodt, *et al*, 1942	Blood	427	2·66	0·31–6·95
	Milk	16	3·14	
	Urine	427	11·81	0·61–34·26
Sampson and Boley, 1945	Blood	49	4·25	0 –7·44

was usually assumed to be β—hydroxybutyric acid. Although qualitative tests for the presence of ketones in milk are frequently referred to, there would appear to have been few quantitative studies. There have been many speculations about the role of the stomach in the etiology of the disease and Boddie (1949) claims to have demonstrated ketone production in the normal rumen, particularly in dairy cows kept indoors on winter feeding. On the other hand, Holmes (1950) using qualitative Rothera tests, could find no evidence of appreciable amounts of acetone or acetoacetic acid in the rumen in cows with ketosis.

It is obvious that the data hitherto available is only of limited value in studying the etiology or pathogenesis of bovine ketosis, in the absence of any accurate differentiation between the various fractions. Moreover, the unsuspected presence of isopropanol in the rumen contents and various body fluids (Robertson, Thin and Stirling, 1950) renders the problem more complicated than has been realised. It was felt worth while, therefore, to re-investigate the condition using methods capable of a more accurate estimation and differentiation of the various fractions comprised under the heading "ketone bodies."

TABLE 2

TOTAL KETONES IN BOVINE KETOSIS

(Mg. Acetone per 100 ml.)

Reference	*Material Examined*	*No. of Samples*	*Mean*	*Range*
Sampson, *et al*, 1933	Blood	40	37·6	12·47–63·83
	Urine	1	80	
Boddie, 1935	Blood	1	10·06	
	Urine	2	77·22	38·12–116·32
Fincher, 1936	Blood	4	55·2	38·7–86·25
	Urine	4	727	570–1025
Sampson and Hayden, 1936 ..	Blood	35	32	10–72·85
	Urine	28	371·1	11–1309·16
Duncan, *et al*, 1939	Blood	40	34·68	4·06–100·0
	Urine	49	395·8	18·3–1309·20
Steyn, 1941	Blood	3	44·23	28·04–56·3
Sampson and Boley, 1941	Blood	8	40·76	21·95–70·93
	Milk	8	16·85	5·46–31·25
	Urine	8	562·09	139·4–1214·9
Sykes, Duncan and Huffmann, 1941	Blood	4	37·7	2·4–89·30
	Urine	4	694·5	338–1568

MATERIALS AND METHODS

Three dairy herds in the vicinity of Edinburgh have been studied during the past two years, milk samples being taken from all the lactating animals at least once a fortnight and every week where possible. These samples were subjected to the following modified Rothera test—two parts of milk were taken in a test tube and mixed with one part of a dry mixture containing 1,000 g. ammonium sulphate, 500 g. anhydrous sodium carbonate and 20 g. sodium nitroprusside. On shaking, samples from clinical cases of ketosis gave a dark coloration almost immediately while subclinical cases gave samples in which the colour took some three to five minutes to develop. In the event of a positive reaction being obtained, samples of blood, urine, milk and rumen contents were collected as soon as possible, the latter being obtained by stomach tube using a suction pump. In addition sporadic samples from clinical cases of bovine ketosis were obtained from veterinary surgeons in many parts of the country. In all these samples the various ketone bodies—acetone, acetoacetic acid, β—hydroxybutyric acid and isopropanol were estimated by methods described elsewhere (Thin and Robertson, 1952). During the period of the investigation a few samples became available from cases of pregnancy toxaemia and these have been similarly studied.

RESULTS

Normal ruminants

Estimations were carried out on blood, rumen contents, milk and urine of normal healthy cows and on blood, rumen contents and urine of healthy sheep with rumen fistulae, on various diets and at different times of the year. The total ketones present varied slightly from day to day and from individual to individual but in all cases examined

TABLE 3

TOTAL KETONES IN NORMAL RUMINANTS

(Mg. Acetone per 100 ml.)

Species	*Material Examined*	*No. of Samples*	*Mean ± S.D.*	*Range*
Cows	Blood	20	6·10 ± 0·59	2·87–10·88
	Rumen Liquor	18	3·26 ± 0·46	0 –10·25
	Milk	18	6·84 ± 1·02	2·87–20·50
	Urine	17	9·54 ± 1·08	4·10–22·55
Sheep	Blood	20	7·43 ± 1·07	0 –12·97
	Rumen Liquor	19	2·93 ± 0·68	0 –10·25
	Urine	13	18·54 ± 3·45	2·05–46·13

only β hydroxybutyric acid could be detected. The ranges and mean values with their standard deviations are shown in Table 3. It will be seen that the levels present in blood and milk were very similar, those in the rumen about half and in the urine nearly twice those of the blood. The values for the sheep were slightly higher than those found in the cow.

Bovine ketosis

In this report are considered the results of analysis of sporadic samples from 89 animals, among which most of the commoner dairy breeds were represented, though the majority were Ayrshire or Ayrshire-cross cows which were the predominant types in the districts from which samples were obtained. Most of the cases occurred in five to six year old animals after their third calving; a large proportion had calved in the months of February or March and symptoms appeared usually some ten to thirty days after parturition, although the range was considerably wider, viz. one to fifty-four days.

In the herds which were being closely followed, it was possible to get material from animals in the early stages of the disease and from subclinical cases. It was possible also to follow a number of animals throughout the whole duration of their illness though only the initial samples obtained are included in this report. The samples sent in by veterinary surgeons were mainly single ones and it was not possible to get samples of all the fluids concerned in every case. They covered a wide range of degree of clinical ketosis and came from animals at different stages in the development of, or recovery from, the disease. It was not surprising, therefore, to find a wide variation in the total ketone content of the samples (Table 4) and the figures for blood encompass most of the values given by earlier workers. The level in milk was about 20 per cent. below that of blood while the rumen liquor had appreciably less; urine on the other hand had a level about six times that of the blood.

TABLE 4

TOTAL KETONES IN BOVINE KETOSIS

(Mg. Acetone per 100 ml.)

	No. of Samples	*Mean ± S.D.*	*Range*
Blood	89	48.06 ± 2.75	10.0 – 116.67
Rumen liquor ..	36	20·06 ± 1·96	2·11 – 55·54
Milk	41	37·35 ± 3.65	9·02 – 122·59
Urine	24	305·77 ± 46·4	32·32 – 757·37

In the case of many of the specimens received from practitioners, we had very little idea as to the stage in the development of the condition at which the samples were taken. In order, therefore, to get some idea of the changes in proportion of the individual ketone bodies during the course of the disease they were divided arbitrarily into groups according to the level of total ketones in the blood (Table 5). Group A (total blood ketones above 60 mg. acetone/100 ml.) included the most severe clinical cases encountered while Group B (30 to 60 mg./100 ml.) also included only clinical cases, mainly of a milder type. All the subclinical cases were found to lie in Group C (10 to 30 mg./100 ml.). It will be appreciated, however, that as clinical symptoms may not become evident until the condi-

TABLE 5

AVERAGE VALUES OF KETONE BODIES IN KETOSIS CASES

(Mg. Acetone per 100 ml.)

Substance and No. of Cases	*Acetone*	*Acetoacetic Acid*	*β-hydroxybutyric Acid*	*Isopropanol*	*Total Ketone Bodies*
A. *Total Blood Ketones above 60 mg. acetone/100 ml.*					
Blood: 28	34·70 ± 2·90	9·04 ± 1·01	30·38 ± 3·98	7·95 ± 0·91	82·07 ± 3·09
Blood: 5	32·58 ± 3·30	10·97 ± 0·96	20·59 ± 4·50	7·84 ± 0·93	72·08 ± 3·22
Rumen: 5	11·75 ± 2·10	2·98 ± 0·77	6·15 ± 2 37	18·42 ± 3·27	39·30 ± 3·94
Blood: 13	37·17 ± 4·19	7·47 ± 1·30	25·69 ± 5·29	8·05 ± 1·19	78·39 ± 4·65
Milk: 13	29·19 ± 3·45	6·99 ± 1·56	19·12 ± 5·98	3·89 ± 0·69	59·19 ± 7·33
Blood: 9	33·52 ± 2·78	9·23 ± 1·94	30·08 ± 6·86	7·09 ± 1·12	79·92 ± 5·28
Urine: 9	58·85 ± 6·27	117·86 ± 14·00	265·78 ± 48·57	38·65 ± 6·83	481·14 ± 63·43
B. *Total Blood Ketones 30–60 mg. acetone/100 ml.*					
Blood: 32	16·92 ± 1·07	4·46 ± 0·59	19·55 ± 1·72	2·23 ± 0·40	43·16 ± 1·55
Blood: 13	17·33 ± 1·96	4·80 ± 0·78	20·45 ± 2·68	2·59 ± 0·76	45·17 ± 2·75
Rumen: 13	7·12 ± 1·03	1·33 ± 0·22	4·51 ± 0·89	9·72 ± 1·32	22·68 ± 2·55
Blood: 18	15·70 ± 1·82	4·32 ± 0·63	19·96 ± 2·26	2·02 ± 0·58	42·00 ± 2·01
Milk: 18	13·54 ± 1·64	2·71 ± 0·41	12·13 ± 2·40	1·24 ± 0·33	29·62 ± 2·63
Blood: 9	15·31 ± 2·32	4·72 ± 1·11	20·52 ± 3·29	2·55 ± 1·03	43·10 ± 3·01
Urine: 9	31·11 ± 4·39	96·12 ± 23·14	125·51 ± 50·10	18·27 ± 10·07	271·01 ± 65·07
C. *Total Blood Ketones 10–30 mg. acetone/100 ml.*					
Blood: 29	5·95 ± 0·99	2·87 ± 0·40	10·66 ± 0·89	0·35 ± 0·19	19·83 ± 0·93
Blood: 18	5·72 ± 1·08	3·79 ± 0·46	10·66 ± 1·23	0·30 ± 0·27	20·47 ± 1·22
Rumen: 18	1·32 ± 0·47	0·66 ± 0·15	6·08 ± 0·98	4·77 ± 0·85	12·83 ± 1·34
Blood: 10	6·77 ± 1·22	3·47 ± 0·68	11·04 ± 1·09	0·51 ± 0·19	21·79 ± 1·68
Milk: 10	5·13 ± 1·40	1·98 ± 0·46	15·08 ± 2·87	0·68 ± 0·27	22·87 ± 4·00
Blood: 6	6·22 ± 1·93	2·45 ± 0·67	11·74 ± 1·60	0·70 ± 0·30	21·11 ± 1·79
Urine: 6	13·28 ± 3·01	42·61 ± 11·26	29·99 ± 5·73	8·99 ± 5·53	94·87 ± 20·32

tion is well advanced, it is possible that certain of the samples submitted by veterinary practitioners were taken when the animals were in the recovery phase.

The rise in total ketone bodies in the blood in these three arbitrary groups was accompanied by a similar rise in rumen liquor, milk and urine samples taken at the same time. (To make the comparisons accurate, the figures for the corresponding blood samples are given every time along with the limited number of samples available of the other fluids.) In each group the rumen liquor contained about half that present in the corresponding blood samples, the milk about three-quarters, except in Group C where blood and milk were practically identical, and the urine about six times the level attained in the blood.

In nearly every case this rise was brought about by an increase in concentration of all the constituent fractions, the only exception being the β–hydroxybutyric acid which, apart from the urine samples, remained comparatively constant, particularly in the milk. In comparing the three groups it will be noted that in Group A acetone was the most prominent constituent in the blood and milk where it formed some 42 per cent and 50 per cent. respectively of the total ketones compared with 30 per cent. and 23 per cent. in Group C. In the urine samples of Group A acetone was a comparatively minor fraction and β—hydroxybutyric acid predominated, comprising more than half the total, as compared with some 30 to 40 per cent. in the other two groups. Acetone had also to take second place to isopropanol in the rumen liquor, comprising less than one-third of the total compared with some 45 per cent. for the latter.

In Group C, β—hydroxybutyric acid was the most prominent constituent in all the materials examined, except urine where it was slightly exceeded by acetoacetic acid. It was also the major fraction in the blood in Group B, though again acetoacetic acid was prominent in the urine, isopropanol in the rumen contents and acetone in the milk.

Apart from the urine samples, the percentage content of acetoacetic acid varied only slightly over the three groups, though the absolute concentration naturally showed a rise with increased severity of the disease. In the urine on the other hand, the percentage content of this fraction fell from nearly half the total to less than one quarter as the absolute amount increased.

The concentration of β–hydroxybutyric acid rose in the blood and urine but varied only slightly in the milk and even less in the rumen liquor. The percentage content of this fraction fell, however, in blood, rumen liquor and milk but rose in the urine.

Both the mean concentration and percentage content of isopropanol rose in all four body fluids by varying degrees, the highest proportion being found in all cases in the rumen liquor.

Pregnancy toxaemia

Only blood samples were available from the fifteen cases of pregnancy toxaemia examined and these were insufficient to warrant division into groups as was done in the bovine ketosis cases. It will be observed, however, from Table 6 that the total ketones present far exceeded in all cases the normal amounts, and was similar to the range of values in the bovine ketosis blood samples. The proportion of the different fractions varied somewhat from those found in bovine ketosis, the acetone and isopropanol concentrations being lower and those of acetoacetic acid and β–hydroxybutyric acid higher.

DISCUSSION

Our figures for ketone body levels in normal cows are, with the exception of those for urine, slightly higher than the results quoted by previous workers which had been obtained by a variety of methods. It may be that the technique used here was more sensitive at lower levels than previous methods and that interfering substances in urine were removed more completely. On the other hand, ketone levels vary considerably from animal to animal and the slight difference noted may be associated with diet, environment, etc. Our values for normal sheep on the other hand are higher than those of Sampson and Boley (1940) and it is doubtful if increased sensitivity in the method of estimation could be the whole explanation. The difference may be associated with the fact that our normal sheep were kept in metabolism stalls and given winter feeding all the year round, with a small supplement of freshly cut grass in summer, while those of Sampson and Boley were kept under normal grazing conditions. There is some evidence of an increase in blood and urine ketones in cows during the winter months (Boddie, 1935; Knodt *et al*, 1942; Holmes, 1950) which has been ascribed sometimes to diet

TABLE 6

BLOOD KETONES IN PREGNANCY TOXAEMIA (15 CASES)

(Mg. Acetone per 100 ml.)

Constituent	*Mean ± S.D.*	*Range*
Acetone	3·97 ± 0·97	0 – 13·50
Acetoacetic acid..	7·53 ± 1·35	1·50 – 17·03
β-hydroxybutyric acid	55·87 ± 9·96	6·03 – 149·73
Isopropanol	0·50 ± 0·12	0 – 1·50
Total ketone bodies	67·87 ± 9·45	23·94 – 152·04

and sometimes to lack of exercise. The same circumstances may account for our sheep figures being higher than those from our normal cows, which included samples from grazing animals as well as from those on winter rations.

The total ketones in our bovine ketosis series cover a greater range than quoted in the literature, again with the exception of the urine samples. In explanation of this, there must be taken into account, in addition to greater sensitivity and specificity of methods, the larger number of animals studied and the inclusion of a number of subclinical cases. But it is when we come to consider the partition of the various fractions that the greatest differences arise between our results and those previously available, which were based usually on quite inadequate methods. It is not surprising, therefore, that our findings do not support many widely held theories about the role of the various fractions and the changes they undergo. Thus the inability to distinguish between acetone and acetoacetic acid has frequently led to the assumption of a higher level of the latter than is shown in our cases; and as most methods involve at some stage oxidation with chromic acid, which oxidises both β–hydroxybutyric acid and isopropanol to acetone, the unsuspected presence of the latter has naturally led to undue prominence being given to the β–hydroxybutyric acid.

Dye and McCandless (1948) suggested that the most important ketone bodies physiologically and clinically were β–hydroxybutyric acid and acetoacetic acid, and ascribed a toxic role to the latter; acetone, it was believed, was formed from these by the body and was inert and ultimately excreted. Now β–hydroxybutyric acid is obviously important physiologically in the ruminant, being the only ketone fraction present in the normal animal and presumably a harmless metabolic product. If, as in other species, acetoacetic acid is formed in the ruminant during fat metabolism, it must be rapidly changed to β–hydroxybutyric acid in the normal animal. Shaw (1942) suggested that the mammary gland could utilise β hydroxybutyric acid for energy purposes and the same may be true for other tissues; the remainder is excreted in the urine.

Our results, however, do not support the view that β–hydroxybutyric acid is important clinically, for, as the severity of the disease increases, the proportion of β–hydroxybutyric acid present decreases except in the urine, where it shows a tendency to increase both in concentration and in relation to the total ketones present. The kidneys show, of course, considerable ability to concentrate this fraction though not so markedly as in the case of acetoacetic acid. From the absence of the latter in the normal state and its low blood levels in both subclinical and clinical ketosis in conjunction with the high levels in the urine, it would seem that it has a low renal threshold which may be associated with its toxic nature.

The presence of large quantities of free acetone in the blood and its excretion in the milk and to a lesser extent in the urine, suggests that this fraction is by no means unimportant but may simply indicate another mechanism for disposing of excessive quantities of acetoacetic acid. Such a chain of events appears to be present in straightforward starvation ketosis of ruminants (Robertson and Thin—in press); and of course a variable amount of anorexia is a usual concomitant of bovine ketosis. Two facts, however, throw doubt on such a simple explanation, viz. the intense degree of ketosis developed, often out of all proportion to the reduction in food consumption, and the relatively high proportion of isopropanol in the rumen contents in the disease.

It seems likely from the starvation studies mentioned above that, when food is withheld, isopropanol is manufactured in the rumen possibly from acetone which diffuses in from the blood stream; the percentage content of acetone in blood and rumen liquor was similar with the percentage of isopropanol in the rumen about half that of acetone. In ketosis, however, the level of acetone in the rumen is considerably lower than in the blood while the isopropanol is much higher and in ketosis the proportion of isopropanol in the rumen is higher than that found in starvation at any level of total ketone bodies. If, therefore, we are to assume a similar chain of events to those postulated in straightforward starvation ketosis, it would appear that in the diseased condition acetone, formed in the tissues from the metabolism of acetoacetate and diffusing into the rumen, produces a different response to that occurring in starvation with the point of equilibrium of a possible acetone⇆isopropanol reversible reaction altered in favour of a greatly increased production of isopropanol. An alternative explanation is that in the disease isopropanol is formed in the rumen from some substance other than acetone and diffuses into the blood stream in part unchanged and in part as acetone, which is then metabolised in the tissues with the production of the other acetone bodies. Thus, though some degree of starvation ketosis must inevitably complicate the picture during severe cases of the disease, it may well be that the above type of reaction plays an important part in the development of the condition. The urinary excretion of ketones in the course of the disease appears, if sporadic samples are any guide, to be much greater than in starved animals with similar blood ketone levels; in addition the proportion of isopropanol is also larger, again emphasising the importance of this fraction.

The range of values covered by the total ketone bodies in pregnancy toxaemia was much the same as in bovine ketosis, but the proportions of the constituent ketone fractions were different. Compared with the bovine disease the concentrations of both acetone and isopropanol were much lower, acetoacetic acid slightly higher and β–hydroxybutyric acid much higher. Very much the

same differences were noted between the responses of the sheep and the cow to starvation. The relative proportions of the ketone bodies thus indicate a close relationship between starvation ketosis in ewes and pregnancy toxaemia, though the percentage values for acetoacetic acid in pregnancy toxaemia are, if anything, slightly higher than those encountered in starvation.

It would appear from comparing the results obtained during starvation ketosis and pregnancy toxaemia that both conditions are similar and caused by the production of excessive quantities of acetoacetic acid from the catabolism of fat. The low concentrations of isopropanol found in the sheep in both starvation and pregnancy toxaemia support the suggestion that the rumen plays a more important part in bovine ketosis than in pregnancy toxaemia.

SUMMARY

Methods devised for accurate differentiation of various ketone bodies have been applied to a study of ketosis in ruminants.

Estimations carried out on blood, rumen liquor, milk and urine from healthy animals revealed only β–hydroxybutyric acid.

Sporadic samples of similar fluids were examined from 89 animals with bovine ketosis, including mild and severe types and subclinical cases; blood samples from 15 cases of pregnancy toxaemia were also analysed.

The partition of ketones in these samples is described and discussed.

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REFERENCES

Boddie, G. F. (1935). *Vet. Rec.*, **51**, 1539; (1949). *Ibid.*, **61**, 85.
Duncan, C. W., Huffmann, C. F., and Tobin, H. A. (1939). *J. Amer. vet. med. Ass.*, **95**, 690.
Dye, J. A., and McCandless, E. L. (1948). *Cornell Vet.*, **38**, 331.
Fincher, M. G. (1936). *Ibid*, **26**, 142.
Holmes, J. (1950). *Personal Communication.*
Knodt, C. B., Shaw, J. C., and White, G. C. (1942). *J. dairy Sci.*, **25**, 851.
Robertson, A., Thin, C., and Stirling, A. (1950). *Nature, Lond.*, **166**, 954.
Sampson, J., and Boley, L. E. (1940). *Cornell Vet.*, **30**, 392; (1941). *Amer. J. vet. Res.*, **2**, 327; (1945). *Cornell Vet.*, **35**, 314.

Sampson, J., Gonzaga, A. C., and Hayden, C. E. (1933). *Ibid,* **23**, 184.
Sampson, J., and Hayden, C. E. (1936). *Ibid.*, **26**, 183.
Shaw, J. C. (1942). *J. biol. Chem.*, **142**, 53.
Shaw, J. C., and Powell, R. C. (1941). *J. dairy Sci.*, **24**, 503.
Steyn, H. P. (1941). *J. South Afric. vet. med. Ass.*, **12**, 86.
Stinson, O. (1929). *Vet. Rec.*, **9**, 115.
Sykes, J. F., Duncan, C. W., and Huffmann, C. F. (1941). *J. dairy Sci*, **24**, 193.
Thin, C., and Robertson, A. (1952). *Biochem. J.*, **51**, 218.

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ENCEPHALITIS IN RACEHORSES IN MALAYA

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INTRODUCTION

A disease producing encephalitic symptoms has been recognised among racehorses in Singapore since 1948. This condition has been probably present in Malaya for a much longer period and the syndrome referred to as "five day fever" (Annual Report, 1947) is in all probability a manifestation of the same disease. In August and September 1951 there was a mild epizootic in Singapore and cases were also reported from the racing stables at Kuala Lumpur about the same time.

CLINICAL TYPES OF THE DISEASE

Approximately 60 racehorses showing evidence of this encephalitic type of disease, have been seen during the last 3 years. Clinically there appear to be three distinct types : (a) transient; (b) lethargic; (c) hyperexcitable.

Transient type. The animal runs a temperature of up to 103°F. for 2 to 3 days, showing congestion of the mucous membrane and sometimes jaundice. The appetite is poor and movements are sluggish but an uneventful recovery is the usual sequel.

Lethargic type. The temperature fluctuates between 102° and 105°F. The appetite is impaired. Swallowing is frequently difficult and in such cases the animal takes a few mouthfulls of chaff or grass, chews them, leaves the food and stands as though drowsy with the head down. After a few minutes "sleep" it wanders round the stall, closing its eyes periodically as if suffering from severe headache.

Jaundice and petechiae of the mucous membranes are usual. A nasal discharge which varies in consistency from a watery to a greenish mucoid exudate is not uncommon and may well account for the difficulty in swallowing. Congestion of the lungs may be expected in cases running a high temperature for any length of time. A most commom symptom is inco-ordination of movement. When in the stall the horse appears to be afraid to move and braces itself with its forelegs apart when standing. If forced to move it will stagger or display high-stepping movements of the fore or hind

legs. On turning it may collapse to the ground but quickly rises again although control of equilibrium is often difficult. Other symptoms lasting up to 36 hours have been transient rigidity of the neck, radial paralysis, hanging of the lower lip and impaired vision of one or both eyes.

In this form of the disease recovery is usual in four or five days and the animal may be back to light work in ten days if no complications, such as congestion of the lungs occur, when a longer convalescence is necessary.

Hyperexcitable form. This is the most spectacular but the least common form, affecting less than five per cent of clinical cases of the disease.

The temperature is elevated for two days and may run as high as 105° or 107°F. The horse appears to go quite "mad" and is almost uncontrollable at times. At the onset, aimless wandering round the stall with the head elevated or lowered to the ground and sudden shying at imaginary objects is common. The eyesight becomes defective; in consequence, superficial damage to the head and other parts of the body results through contact with the walls of the stall. Sweating is profuse and whole groups of muscles throughout the body are seen to twitch spasmodically. Bouts of grinding of the teeth suggestive of severe pain are often noticed. At the height of the symptoms affected horses are liable to crash through the bars of the stall or injure themselves extensively if restricted to the confines of a normal stall. When on a long rein the horse will not stand still but will circle at a walk or trot; every now and again it may rear, stagger or fall over backwards. The severe symptoms last only a short time and often disappear in eight hours. In cases of this type death is not uncommon.

LABORATORY INVESTIGATIONS

Post-Mortem Findings

Only 4 of the 60 horses seen during the 3 years died or were destroyed and only one of them was examined post-mortem. This horse, a four year old English thoroughbred gelding had been nine months in Malaya when it developed a typical encephalitis of the lethargic type. Recovery appeared normal but three months after the initial infection incoordination of movement became apparent and in a further three months the animal was paraplegic. For humane reasons the horse was destroyed.

The only macroscopic lesions seen were areas of congestion in both lungs. The brain was removed and sections of the pons and cerebellum showed typical lesions of encephalitis. There was a microglial and round cell infiltration, evidence of nerve cell degeneration, marked perivascular lymphocytic infiltration (Figs. 1, 2 and 3).

At this time no attempts at virus isolation were made.

Serological Findings

The presence of neutralising antibodies to Japanese type B encephalitis virus in horse sera was carried out by the intracerebral inoculation technique (Diagnostic Procedures, 1948). Complement fixing antibodies were estimated by the method of Casals and Palacios (1941).

The sera of five horses which had undoubtedly suffered from this type of encephalitis 10 months previously were tested for the presence of antibodies to the Japanese B virus. At this stage all gave a negative complement-fixation reaction but neutralising antibodies were present and the neutralisation index in all was over 100. Samples of sera were taken from a horse, at nine days and six weeks after the onset of the hyperexcitable type of encephalitis. The first sample was negative to the complement fixation test but gave a neutralising index of 3,400. Six weeks after the onset of the illness the serum gave a complement fixation titre of 1/32.

In view of these findings about thirty samples of sera from horses which had showed no overt symptoms were tested for the presence of neutralising antibodies. Twenty horses demonstrated neutralising antibodies in their sera. Since these results were obtained the survey has been widened and it is hoped to publish the results in a further communication.

DISCUSSION

The mortality rate in horses from this disease was very low, the chief loss being due to interference with the racing career of the animal involved. This and the fact that sick animals were never sacrificed owing to their commercial value prevented access to material suitable for isolation of the aetiological agent. The presence of the neutralising antibodies in the sera of horses which were affected but had recovered was suggestive, however, that the infecting agent was the Japanese encephalitis type B virus. This suggestion was very much strengthened by the demonstration of a rising titre of complement fixing antibodies in a horse following the onset of encephalitis.

Japanese type B encephalitis virus was isolated from human cases of encephalitis in Malaya (Hale, Lim and Chee, 1952). Neutralising antibodies to the Japanese B virus have been found in sera of horses in countries where Japanese B Encephalitis is endemic (Sabin, 1947; Bawell, Deuel, Matumoto and Sabin, 1950; Deuel and Sabin 1950). The situation in Malaya is apparently similar and if horses are infected with Japanese type B encephalitis virus they may act as a reservoir of infection. Although many horses in Malaya have shown no clinical signs of disease, the results so far obtained show that a high proportion have neutralising antibodies in their sera, indicating that the infection is very widespread.

Nevertheless a more complete serological survey of the Malayan horse population must be completed before it is possible to assess the importance of this population as a reservoir of infection.

All racehorses are imported and no breeding takes place in Malaya. This raises the possibility of the introduction of other strains of equine encephalitis virus into the country. Australia exports racehorses to Malaya and it has been shown that a high proportion of horses in the northern part of State Victoria have neutralising antibodies to Murray Valley Encephalitis virus (Anderson S.G.) which indicate past infection. Similarly the export of racehorses from Malaya may introduce the Japanese Encephalitis virus to countries at present free from this infection. The importing or exporting of virus strains will obviously depend upon the distance horses are transported and the method of travel. Prolonged sea journeys will usually result in any infected horses recovering or dying before arrival at their destination, but the use of air travel renders possible the introduction of horses with frank clinical signs which are potentially infective.

SUMMARY

The clinical symptoms and post-mortem findings in encephalitis in Malayan racehorses are described.

Serological evidence implicates the Japanese type B encephalitis virus as the aetiological agent.

REFERENCES

Anderson, S. G. *Walter and Eliza Hall Institute for Medical Research* (personal communication).

Bawell, M. B., Deuel R. E., Matumoto M., and Sabin A. B. (1950). *Amer. J. Hyg.*, **51**, 13.

Casals, J., and Palacios R. (1941). *J. exp. Med.*, **74**, 409.

Deuel, R. E., and Sabin, A. B. (1950). *Amer. J. Hyg.*, **51**, 13.

——— (1948). *Diagnostic Procedures for Virus and Rickettsial Diseases.* American Public Health Association, p. 200.

——— (1947). *Direct. Vet. Services, Malaya,* Annual Report.

Hale, J. H., Lim, K. A., and Chee, P. H. (1952). *Ann. trop. Med. Parasit.* **46**, 220.

Sabin, A. B. (1947). *J. Amer. med. Ass.*, **133**, 281.

(*Received for publication, December 30th,* 1952)

Fig. 1

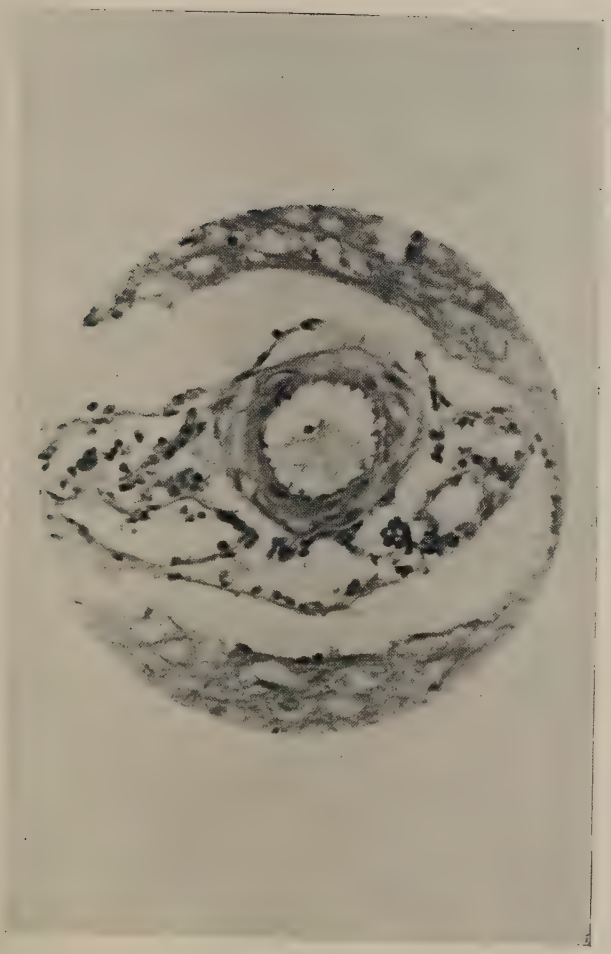

Fig. 1. Perivascular infiltration Cerebellum X364.

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Fig. 2

Fig. 2. Perivascular infiltration Pons X280.

Fig. 3

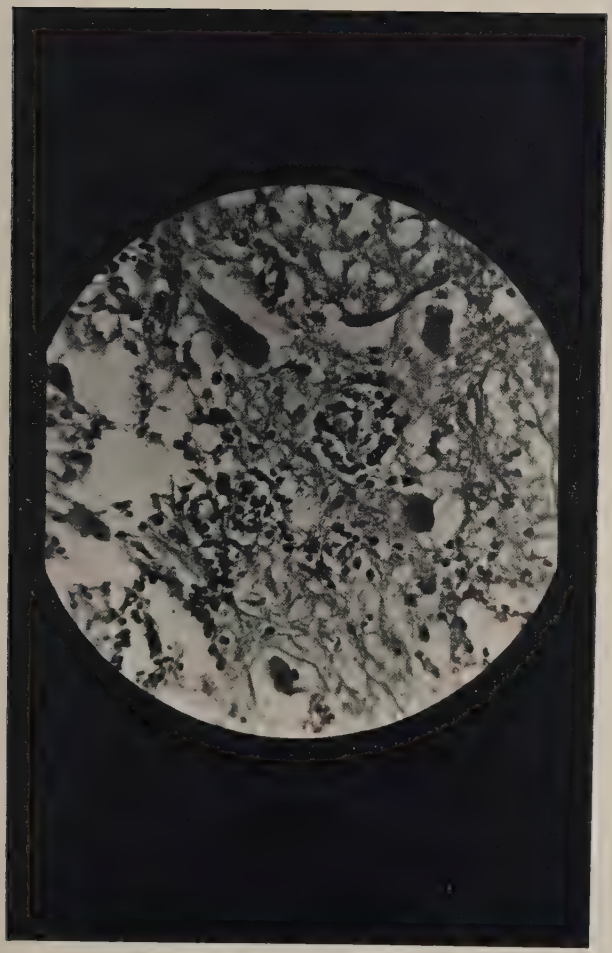

Fig. 3. Pons showing neuroglial and round cell infiltration and commencing neuronal degeneration. X280.

EXPERIMENTAL MASTITIS PRODUCED IN GOATS BY *STREPTOCOCCUS DYSGALACTIAE*

By

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INTRODUCTION

In 1949, after preliminary work in mice, a series of studies was carried out on the experimental mastitis produced in goats by infection with a strain of *Str. agalactiae*. These studies (Pattison, Taylor and Holman, 1950; Taylor, Holman and Pattison, 1950; Holman, Pattison and Taylor, 1950; Pattison and Holman, 1951; Pattison, 1951, 1952; Holman, Pattison and Gordon, 1952) made it clear that the experimental disease produced in goats was similar to the naturally occurring bovine disease.

Using the same methods, this paper extends the investigation to a strain of *Str. dysgalactiae*, obtained from a natural case of bovine mastitis, so that the disease produced by intramammary inoculation of goats with this organism could be contrasted and compared with that produced by *Str. agalactiae* in the same species.

TABLE 1

RESULTS OF INOCULATING GROUPS OF 10 MICE INTRAPERITONEALLY

(0·5 ml. of culture of 19 different strains of *Str. dysgalactiae* each grown for 24 hours in Todd-Hewitt broth)

Research centre supplying strain	*Strain*	*Viable count of organisms: 10^6/ml.*	*Deaths out of ten mice; days after inoc.*			*Total deaths*
			1	2	3	
Compton	B51	100	0	0	0	0
Mr. Neave, N.I.R.D.	273	850	0	0	0	0
" "	274	300	0	0	0	0
" "	276	300	1	0	0	1
" "	278	200	0	0	0	0
" "	283	1350	0	0	0	0
" "	287	250	0	1	0	1
Mr. Wilson, Weybridge	8747	350	0	0	0	0
" "	09	250	0	0	0	0
" "	06	150	0	0	1	1
" "	419	1550	2	1	1	4
" "	113	250	0	0	1	1
" "	115	250	0	1	0	1
" "	C5	300	0	0	0	0
" "	5	600	0	1	0	1
" "	50/42	350	0	1	1	2
" "	50/70	200	0	2	1	3
" "	169	300	0	2	0	2
Dr. Kane, Cooper Tech. Bureau	4E	900	0	0	0	0

MATERIALS AND METHODS

Strain selected. Nineteen strains of *Str. dysgalactiae* were obtained through the kindness of other research laboratories and after preliminary tests, which included a test for pathogenicity in mice as detailed in Table 1, strain 419 obtained from the Ministry of Agriculture and Fisheries, Veterinary Laboratory, Weybridge was selected for further investigation.

Strain 419 showed the essential characteristics ascribed to *Str. dysgalactiae* by previous workers (Minett, Stableforth and Edwards, 1929; Diernhofer, 1932; Edwards, 1932; Little, Brown and Plastridge, 1946). It produced acid in glucose, lactose, sucrose and trehalose, but not in mannitol inulin, aesculin, sorbitol nor raffinose; it did not hydrolyse sodium hippurate; acid, clot and slight reduction were produced in litmus milk; methylene blue milk 1:1000, 1:5000 and 1:20,000 was clotted and reduced at 48 hours; the pH in 0·5 per cent. glucose broth after five days' incubation at 37°C. was 5·0; and serologically the strain belonged to Lancefield group C. The appearance of colonies on bactotryptose ox-blood agar can be seen from Figs. 4 and 5.

TABLE 2

RESULTS OF INOCULATING MICE INTRAPERITONEALLY WITH DIFFERENT DOSES OF *STR. DYSGALACTIAE*

(Strain 419 grown overnight in Todd-Hewitt broth)

Dose of culture	*No. of experiments*	*No. of mice inoculated*	*Total deaths in three days*	*Deaths per cent*
0·25 ml.	20	200	72	36
0·5 ml.	3	30	17	56
1·0 ml.	14	223	208	93
1·5 ml.	2	20	20	100

Further tests were carried out to confirm the pathogenicity for mice of strain 419 and the results are summarised in Table 2; the intraperitoneal dose of 0·25 ml. was chosen as a test dose for mice. The frequency distribution for the number of mice killed is shown in the first line of Table 6.

Plan of investigation

First experiment. The investigation was carried out in two experiments. In the first experiment the largest dose of living organisms was intended to overcome any natural resistance to infection and to ensure the production of measurable pathological changes. Further doses in a diminishing series were tested in order to permit the selection of a suitable test dose in subsequent work. To differentiate the changes caused by the presence of the live organism from those that might be produced by inoculation into the udder of other foreign material, similar volumes of (i) killed organisms (ii) a filtrate from living culture (iii) the culture medium alone, were each injected into control goats : the details of these inoculations are recorded in Table 3.

As in the case of preliminary experiments with *Str. agalactiae*, all goats in this experiment were slaughtered nine days after inoculation to provide a histological comparison between the lesions produced by the two different organisms.

Second experiment. The second experiment was carried out to obtain material for histological examination from animals killed at different intervals after inoculation and to observe the course of the disease beyond the nine-day limit set by the first experiment.

The test dose selected as a convenient one after the first experiment was one of 10^9 organisms, which was the number calculated as present in 1 ml. of a 20-hr. Todd-Hewitt broth culture. This volume was adjusted to 10 ml. by the addition of Todd-Hewitt broth before it was inoculated. Six goats were inoculated and these were slaughtered during a period extending from 1 to 64 days, as detailed in Table 3.

Methods

The bacteriological, biochemical and haematological methods were those described by Pattison *et al.* (1950) and by Pattison and Holman (1951). Agglutination tests, using strain 419 as antigen, were carried out by the method described by Holman *et al.* (1952).

Recordings

Measurements were recorded for at least seven days before the goats were inoculated.

In the first experiment, body temperature and the yield of milk from inoculated and non-inoculated sides of the udder were recorded twice daily; bacteriological cultures of milk from both sides of the udder were also made twice daily. Morning milk samples from both sides of the

TABLE 3

INTRAMAMMARY INOCULATIONS

(Each administered as a volume of 10 ml. through the teat canal on one side of the udder of 14 goats, of (a) graded doses of a 20 hr. culture of *Str. dysgalactiae*, strain 419 grown in Todd-Hewitt broth and (b) control substances.)

Experiment No.	*No. of goat*	*Inoculated with*	*Time killed after inoculation: days*
1	1	Todd-Hewitt broth only	9
	2	Seitz EK filtrate—broth culture—strain 419	"
	3	10^{11} heat-killed strain 419	"
	4	10^{11} living strain 419	"
	5	10^9 " " "	"
	6	10^7 " " "	"
	7	10^5 " " "	"
	8	10^3 " " "	"
2	9	10^9 living strain 419	1
	10	" " " "	2
	11	" " " "	4
	12	" " " "	16
	13	" " " "	31
	14	" " " "	64

udder were examined daily for neutrophil content, pH, chloride content and sediment volume, and were examined by the Whiteside test. Agglutination tests of milk whey from both sides of the udder were made once daily. Blood samples were taken for leucocyte counts several times on the day of inoculation, twice on the second day, and once daily thereafter until the goat was destroyed or until the count had been normal for some days. The agglutination titre of blood serum for strain 419 was examined before inoculation and at the end of the experiment on the ninth day. Heart, lungs, liver, spleen, and kidneys, were cultured at the end of the experiment, and where the goats had been inoculated with live organisms the supramammary lymphatic glands from both sides of the udder were also cultured.

In the second experiment the less important tests on milk were omitted. Body temperature and the yield of milk from inoculated and non-inoculated sides of the udder were measured twice daily. Bacteriological examination of the milk was made daily for 14 days and thereafter on every fifth day until the end of the experiment; milk neutrophil counts from both sides of the udder were made at the same time. Blood leucocyte counts were made at frequent intervals on the day of inoculation and were continued at wider intervals until the count had returned to within the normal range. Heart, lungs, liver, spleen, kidneys, and supramammary glands were cultured at the end of the experiment.

RESULTS

Reaction to inoculation of sterile Todd-Hewitt broth

No reaction was detected.

Reaction to inoculation with sterile Seitz Ek filtrate

A slight febrile reaction was accompanied by a detectable swelling on the inoculated side.

The milk yield dropped, and this change was more pronounced on the inoculated side, the milk from which gave a positive reaction and contained a high proportion of neutrophils for a few days. No other abnormalities were recorded except for a low blood leucocyte count, with a shift to the left, at 24 hours.

Reaction to inoculation of heat-killed organisms

This resembled in degree the reaction obtained with the filtrate.

Reaction to inoculation of live organisms

Working with *Str. agalactiae*, Pattison and Holman (1951) observed that the quickness of onset of the initial systemic reaction was dependent on the number of live organisms inoculated, but that the severity of the mastitis varied among goats and might be marked, intermediate, or minimal. Thus a dose of many million organisms would produce a sharp systemic reaction within six hours but in some animals the mastitis would be minimal and there would be little loss of milk. In contrast, 1,000 living organisms might not produce a clinical response until the third day but if the

goat were susceptible the resultant mastitis might be marked and the milk yield of the inoculated side of the udder reduced to a few millilitres.

Very similar results were found in experiments on goats inoculated with *Str. dysgalactiae*. Table 4 demonstrates the reaction shown by an animal in the first experiment and also records the results for all the tests over a period of nine days for a direct comparison with the work published on *Str. agalactiae*. Figure 1 records the more important measurements over a longer period, to show the difference between marked and minimal mastitis in two goats receiving the same dose in the second experiment.

Fig 1.

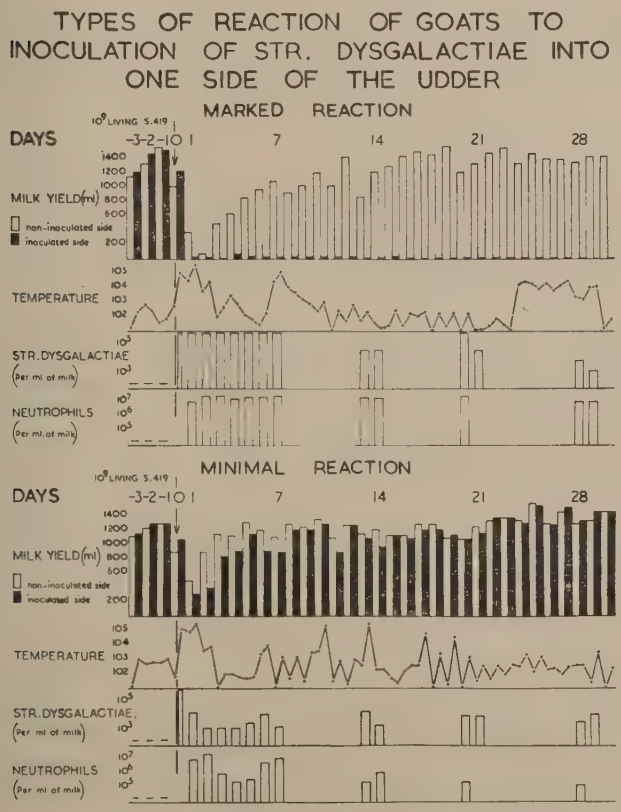

Fig. 1. Marked and minimal reactions in two different goats to intramammary inoculation of the same number of *Str. dysgalactiae* strain 419.

TABLE 4

REACTION TO THE INOCULATION OF 10 ml. TODD HEWITT BROTH CONTAINING 10^{11} VIABLE *STR. DYSGALACTIAE*, STRAIN 419, INTO THE TEAT CANAL OF ONE SIDE OF THE UDDER

Measurement	*Days before inoculation*				*Days after inoculation*																	
	−2		−1		+1		+2		+3		+4		+5		+6		+7		+8		+9	+10
	a.m.	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*a.m.*
Milk yield (ml.) :	540	360	540	360	630	180	10	10	30	10	60	40	45	45	45	45	45	10	30	10	30	20	10
Whiteside (units) :	0		0		0		4		4		4				4		4		4		4		4
Sediment volume (%) :	<0·1		<0·1		<0·1		<0·1		1·3						0·4		0·2		1·3		0·3		
pH :	6·70		6·70		6·70		7·20		7·59		7·60				5·90		7·00		6·21		7·00		7·40
Chlorides (mgm./100ml.) :	146		158		151		432		331		779				265		192		277		288		288
Milk neutrophils (10^6/ml.) :	0		0		0	3·0	26·2		37·2		49·6		32·8		38·0		41·2		52·0		45·6		34·4
Blood leucocytes (10^3/cmm.) :	7·0		12·7		6·6	1·8	2·2	1·8	4·2		6·4				8·0				14·7				6·4
Metamyelocytes (%) :	1		0		0	0	18	4	6		5				9				12				0
Neutrophils (%) :	15		22		30	10	22	12	9		6				11				14				48
Monocytes (%) :	1		3		8	20	13	20	23		33				9				5				4
Lymphocytes (%) :	80		75		61	70	45	64	54		56				69				49				48
Eosinophils (%) :	3		0		1	0	2	0	4		0				1				2				0
Basophils (%) :	0		0		0	0	0	0	4		0				1				0				0
Haemoglobin (gm./100ml.) :	12·0		12·7		13·0	11·5	10·9		11·2						11·7				9·0				9·0
Temperature (°F over 100)	0·8	3·0	1·4	2·4	2·4	3·9	1·4	3·8	2·0	2·6	1·8	3·4	5·0	4·0	5·8	3·4	3·6	5·5	6·0	5·4	5·0	4·4	4·0
Organisms (10^3/ml.) :	0	0	0	0	0	100	100	100	100	37	100	100	100	100	100	100	100	100	100	100	100	37	37
Systemic Reaction :	None				dullness; inappetence; brighter, soft fever; diarrhoea; hiccups														dull again and inappetence, lethargy				
Local reaction :	None				slightly swollen with swelling of lymph node														swollen and diffusely indurated and lymph node very enlarged				

Time of first systemic reaction. The goat receiving 10^{11} living organisms (Table 4) reacted within 6 hours. In the case of animals inoculated with 10^{9} organisms (one goat in the first experiment and six in the second) both rapid and delayed reactions were observed. Where the dose was 10^{3} or 10^{5} the reaction was not observed until 48 hours after infection.

Clinical reaction. The initial systemic response to inoculation varied with the dose and was most obvious in the goat having the largest dose. In this goat (Table 4) dullness was obvious six hours after inoculation and was accompanied by raised temperature, inappetence, diarrhoea, hiccuping, and slight swelling and heat of the inoculated side of the udder. Dullness persisted for the following two days, but there was an improvement on the third day. On the fourth day the animal was very dull again, but improved slightly towards the end of the experiment. The inoculated side of the udder in this animal showed progressive swelling and diffuse induration over the nine days of the experiment accompanied by marked swelling of the supramammary lymphatic gland of the inoculated side. Several temperatures well above the normal range were recorded at irregular intervals over the nine-day experimental period.

With a dose of 10^{9} organisms the initial systemic reaction varied from animal to animal and the common symptoms were a rise in temperature accompanied by lethargy, inappetence and diarrhoea. This reaction usually lasted two days and was followed in the majority of cases by complete recovery; in some animals occasional brief periods of dullness were noted throughout the experiment. With the smaller doses symptoms were limited to a slight rise in temperature accompanied by lethargy appreciable about 48 hours after inoculation.

The period of initial systemic disturbance was characterised by a sharp fall in total blood leucocytes, caused by an absolute decrease in neutrophils; differential counts showed a well marked shift to the left.

Mammary gland and milk-yield. The yield from the non-inoculated side of the udder was always reduced for 48 hours after the inoculation of the other side, and then returned to normal. The severity of the clinical reaction in the inoculated side varied from goat to goat and was closely related to the milk yield; when the udder was diffusely swollen and indurated the yield was always small. In some cases swelling was slight and the induration was limited to a small area near the supramammary lymphatic gland. Progressive enlargement of the supramammary gland on the inoculated side of the udder was a marked feature of the disease. (Fig. 3). The difference between a rapid and permanent milk loss and a slight loss is shown in figure 1.

Tests on milk samples. In the first experiment, chloride, pH, and sediment values for milk from inoculated sides varied widely among animals, and there was no consistent correlation between abnormalities as suggested by these tests and the number of living organisms inoculated. Whiteside tests on the milk from inoculated sides of all animals were positive throughout the nine-day experimental period. In some goats the Whiteside test on milk from non-inoculated sides was occasionally weakly positive. Counts of neutrophils in milk were abnormally high in all inoculated sides shortly after inoculation, and at this time these cells were sometimes found in small numbers in the milk from non-inoculated sides. As the disease progressed, abnormal milk neutrophil counts from inoculated sides tended to fluctuate from day to day, and varied widely from individual to individual; high counts were frequently related to sudden increases in the number of streptococci excreted and to transitory rises in body temperature.

BACTERIOLOGICAL FINDINGS

Recovery of Str. dysgalactiae

Examination of milk samples. Str. dysgalactiae became established in every udder inoculated with the living organism and persisted throughout the experiment. For the first day, 0·02 ml. of milk spread on a 4 inch plate gave innumerable colonies but later the colonies were often countable. Milk samples from the inoculated side of the three control goats and the non-inoculated sides of all goats were also examined but *Str. dysgalactiae* was not recovered.

Examination of body tissues. After slaughter the heart-blood, liver, kidney, and spleen were cultured but on no occasion was *Str. dysgalactiae* recovered. Where goats were inoculated with the live organism the supramammary gland from each side was also cultured and, in the goat killed 16 days after inoculation, one colony, which could not be distinguished biochemically or serologically from *Str. dysgalactiae* strain 419, was isolated from the supramammary gland of the inoculated side.

Agglutination reactions

With *Str. agalactiae,* strain S13, no agglutination with either serum or whey was obtained from goats that had not been experimentally infected (Holman, *et al.*, 1952). This was not the case with *Str. dysgalactiae* strain 419, and with the antigen we prepared, no titre below a definite agglutination at 1:8 was considered positive.

The reactions are recorded in Table 5 and illustrate the following points. Firstly, in many goats the whey titre became positive before the blood serum; secondly, a positive titre could be succeeded by a negative one; thirdly, in two goats there was a positive titre in the non-infected side of the udder.

TABLE 5

RESULTS OF AGGLUTINATION TESTS ON BLOOD SERUM AND MILK WHEY OF GOATS USED IN EXPERIMENTS 1 AND 2

Goat No.	*Tests after inoculation*	*Blood Serum*		*Whey from inoculated side*	
		First titre	*Later titres*	*First titre*	*Later titres*
1	9	None	None	None	None
2	9	None	None	None	None
3	9	None	None	None	None
4	9	1:8 on day 9	..	1:8 on day 8	1:64 on day 9
5	9	1:8 on day 7	1:8 on day 9	None*	None
6	9	None	None	1:8 on day 7	Pos. 1:32 on day 9
7	9	None	None	1:16 on day 7†	1:16
8	9	None	None	1:8 on day 7	1:8
9	1	None	None	None	None
10	2	None	None	None	None
11	4	None	None	1:8 on day 4	..
12	9	1:16 on day 9	Pos. 1:16 on day 16	1:8 on day 10	Neg., then up to 1:8 on day 16
13	12	1:8 on day 13	Pos. up to 1:32 on day 31	1:8 on day 4	Pos., up to 1:32 on day 31
14	15	1:8 on day 3	Pos. up to 1:32 on day 11, then down to neg. on day 16.	None	None

*Goat 5 gave in whey from non-inoculated side, a titre of 1:16 on day 6, 1:8 on day 7, then neg, on day 8 and 9.
†Goat 7 gave in whey from non-inoculated side a titre of 1:8 on day 8, then neg. on day 9.
The treatment of individual goats is given in Table 1.

The results are somewhat similar to those obtained for *Str. agalactiae* and indicate that there is usually some detectable antibody by the end of the ninth day after infection.

Occurrence of crises and increase in virulence

Holman, *et al.* (1952) drew attention to the occurrence of crises following infection with *Str. agalactiae*. Similar crises were found to occur with *Str. dysgalactiae* and figure 2 records a crisis occurring in the goat that received 10^5 organisms. It can be seen that the rise in number of organisms in the milk, recorded on the morning of the fourth day, was followed by a rise in temperature and a fall in milk yield the same afternoon, and that succeeding tests showed a further decrease in yield together with a rise in milk neutrophils.

In the case of both *Str. agalactiae* and *Str. dysgalactiae* it was assumed that some periods of crisis were overlooked owing to their occurrence during the night when temperatures were not recorded. In spite of this there were sufficient data in experiments with *Str. agalactiae* to prove a loose association between the observation of crises and an increase in mouse killing power of organisms subsequently recovered from the milk of the infected goat. In the first experiment with *Str. dysgalactiae* the mouse killing power was tested and the results, recorded in Table 6, show very clearly that there was an increase in killing power during the experiment, but there was insufficient evidence to prove any association between this increased virulence and the observation of a crisis.

Fig. 2

PERIOD OF CRISIS— GOAT INOCULATED WITH LIVING STR. DYSGALACTIAE

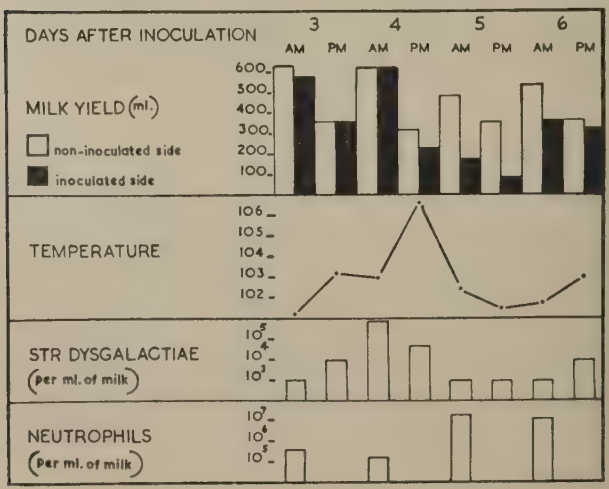

TABLE 6

CORRELATION TABLE SHOWING THE DEATH RATE IN GROUPS OF 10 MICE

(Each received an intraperitoneal dose of 0·25 ml. of S 419 grown overnight in Todd-Hewitt broth. Comparing: (a) tests made at various times on subculture of S419 *in vitro* : (b) tests made on strains of S419 recovered from the milk of five goats at different times during the first experiment.

Inoculum for mice	*Day of experiment*	*Number of tests*	*Deaths out of 10 mice, frequencies*										
			0	1	2	3	4	5	6	7	8	9	10
Stock strain 419	—	25	7		13			4			1		
Strains isolated from milk of infected goats	1/2	12	4		4			4			0		
	3/4	17	4		8			3			2		
	5/6	15	1		6			8			0		
	7/8	15	0		6			6			3		
	9	14	0		0			7			7		
		98											

r = ·53, p less than ·001

Occurrence of variants of strain 419

Observation over many months of the growth characteristics of *Str. dysgalactiae* strain 419 showed that this organism produced small round, greyish, colonies on the surface of Bactotryptose ox-blood agar plates after incubation for 24 hours at 37°C.; these colonies were non-haemoloytic or with a narrow zone of greenish discoloration which became surrounded by a zone of clearing on refrigeration. True beta haemolysis after incubation for 24 hours at 37°C. was not seen in the course of routine subculture in the laboratory.

When strain 419 was inoculated into the udders of goats, however, two variants were encountered in primary isolation from milk spread on the surface of Bactotryptose ox-blood agar plates and incubated for 24 hours at 37°C. The more common of these was a small colony variant, as shown in figure 4, and the other a beta-haemolytic variant, as shown in figure 5. Neither of these could be differentiated biochemically or serologically from *Str. dysgalactiae* strain 419.

DISCUSSION

The results obtained by infecting the udders of goats with *Str. dysgalactiae* strain 419 are best summarised by saying that they bear a striking similarity to those previously obtained for *Str. agalactiae*, strain S 13. When either organism was introduced into the udder in massive doses a systemic effect was produced in an hour or two, while with smaller doses the systemic effect was delayed for several hours. The resultant mastitis could be marked, intermediate or mild irrespective of the size of the infecting dose.

The numbers of organisms in the milk varied according to unknown circumstances and in some goats increased to high numbers to produce a "crisis" which was associated with a high body temperature, a fall in milk yield and an increase in milk leucocytes in the infected gland. The histological picture of the mastitis produced by strain 419, described elsewhere (Pattison and Smith, 1953) was indistinguishable from that produced by *Str. agalactiae*. In marked reactors all the indirect tests on milk became positive, the alteration in pH being the least reliable, and the infected gland was reduced to a fraction of its former size. With intermediate or mild reactors the indirect tests, with the exception of the milk leucocyte count, were quite unreliable.

During infection with either organism there was a definite increase in the mouse killing power of a proportion of strains recovered from the milk of infected glands; variant strains, distinguished by colony size or alteration in haemolytic power, were also isolated. Both *Str. dysgalactiae* and *Str. agalactiae* produced agglutinins which were detectable in the blood serum or in the whey from the infected gland, or rarely, from the non-infected side.

CONCLUSION

Mastitis was induced in goats by the intramammary injection of *Str. dysgalactiae*. The clinical and haematological changes, the alterations in the milk detectable by biochemical tests, and the histological changes in the tissues, were all indistinguishable from those produced by *Str. agalactiae*. As with *Str. agalactiae*, the organisms persisted in the udder throughout the experiment and produced agglutinins that could be detected in the blood serum and in the whey by using a plate test.

ACKNOWLEDGMENT

We wish to thank Mr. F. H. Summerfield for the photographs.

REFERENCES

Diernhofer, J. (1932). *Z. Haust.*, **42**, 67. (cited by Minett, F. C. (1934) *XII Int. Vet. Congress*, **2**, 511).
Edwards, S. J. (1932). *J. comp. Path.*, **42**, 43.
Holman, H. H., Pattison, I. H., and Taylor, J. I. (1950). *Ibid.*, **60**, 100.
Holman, H. H., Pattison, I. H., and Gordon, W. S. (1952). *Ibid.*, **62**, 196.
Little R. B., Brown, J. H., and Plastridge, W. N. (1946). In Little and Plastridge. *Bovine Mastitis*. New York.
Minett, F. C., Stableforth, A. W., and Edwards, S. J. (1929). *J. comp. Path.*, **42**, 213.
Pattison, I. H. (1951). *Ibid.*, **61**, 71; (1952). *Ibid.*, **62**, 1.
Pattison, I. H., and Holman, H. H. (1951). *Ibid.*, **61**, 26.
Pattison, I. H., and Smith, I. M. (1953). *J. Path. Bact.*, In press.
Pattison, I. H., Taylor, J. I., and Holman, H. H. (1950). *J. comp. Path.*, **60**, 71.
Taylor, J. I., Holman, H. H., and Pattison, I. H. (1950). *Ibid.*, **60**, 92.

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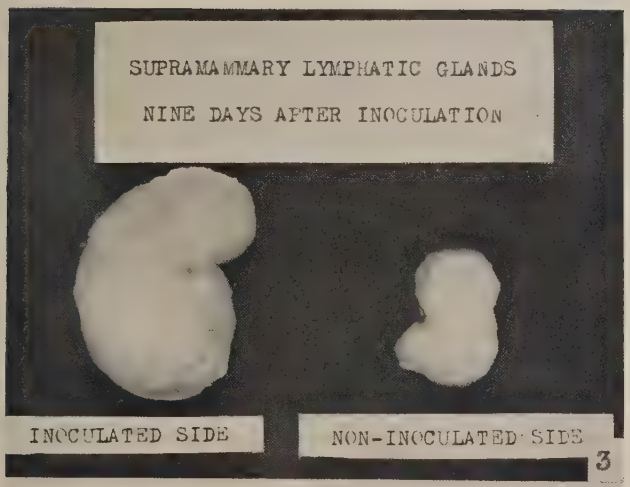
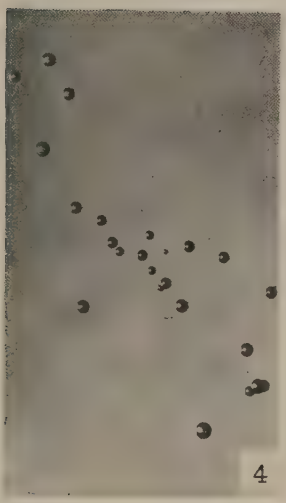
Figs. 3, 4 & 5





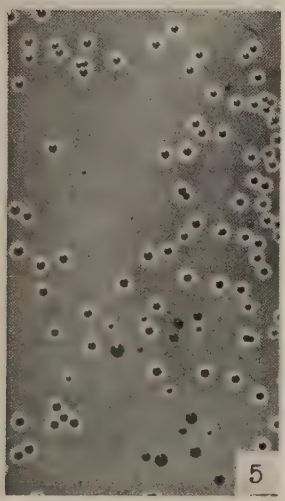


Fig. 3. Enlargement of the supramammary lymphatic gland nine days after intramammary inoculation of *Str. dysgalactiae* strain 419.

Figs. 4 and 5. Small colony and haemolytic variants of *Str. dysgalactiae* strain 419 after growth in the udder. In each figure typical colonies of the organism are also present.

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FURTHER OBSERVATIONS ON CAESARIAN SECTION IN THE BOVINE

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INTRODUCTION

In a previous paper eighteen cases of bovine caesarian section were recorded (Wright, 1949). In this communication 45 additional cases dealt with by the clinical staff at the Field Station of the School are discussed: 39 of them were attended at the request of practising veterinary surgeons and were transported to the Station from distances up to 70 miles while in labour. In these delay after the onset of dystocia was a constant feature. Its degree, however, varied from five or six hours to several days; moreover, the degree and duration of interference *per vaginam* before operation was also variable. These factors, while greatly detracting from the results, are largely inseparable from the work of an animal hospital. The staff of the Station were in control from the outset in six cases only (Nos. 4, 7, 10, 14, 36 and 39). In three of these, operation was a preparturient measure and the others were early dystocia cases. The animals involved in the whole series comprised 26 first-calf heifers and 19 cows which had had one or more previous calves. The breeds concerned were: Friesian—23, Friesian x Shorthorn—3, Shorthorn—7, Ayrshire—9 and Guernsey—2. These figures are not proportional to the respective breed populations in the area from which they were received. Insofar as Cheshire is concerned these may be taken as Ayrshire first with Friesian only a little behind. The Shorthorns were received chiefly from North Wales. There are relatively few of the Channel Island breeds in the district.

RESULTS

Recovery rate. The overall maternal recovery rate was 62 per cent. On reviewing the case protocols it becomes clear that the most important single factor in regard to the outcome of operation for the mother was the time after the onset of the second stage of labour at which operation was performed. In many cases, however, it was impossible to obtain reliable information on this score. If the fact that the calf was alive at the time of operation is taken as the indication of a recent case, then the maternal recovery rate was 76·5 per cent. Insofar as normal calves are concerned, of 14 which were alive when removed from the uterus, 12 survived, one died shortly after delivery and the other two days after operation. Three cases of *Schistosomus reflexus* were dealt with in which the foetal heart was beating when the monster was removed from the uterus; the three

mothers survived. As far as could be ascertained the duration of second stage labour in all cases in which the calf was alive was not more than twelve hours. In the 25 cases in which the calf was dead at the time of operation (this is omitting three cases in which operation was a preparturient one) 13 mothers recovered (52 per cent). There was, of course, great variation in the degree of delay in the latter group; some had been in labour for at least 48 hours by which time the foetus and the foetal membranes were in a state of advanced putrefaction and the animal severely ill.

INDICATIONS FOR OPERATION

Foetal oversize. In this group there were 14 cases comprising 33 per cent of the total. The breeds involved were Friesian—10 (9 heifers, 1 cow), Shorthorn—2 (1 heifer, 1 cow), Ayrshire—1 (heifer) and Guernsey—1 (cow). An additional factor in many was that there was an almost complete loss of foetal fluids and the uterus was contracted on the irregular contour of the foetus.

Developmental abnormalities. There were 14 cases (33 per cent) in this group. The conditions encountered were as follows :

Schistosomus reflexus—6. Friesian 2, (cow and heifer) ; Friesian x Shorthorn 2 (cow and heifer) ; Shorthorn 2 (heifers). One case was further abnormal in that the monster had five fully-developed limbs. (Figs 1 and 2).

Achondroplasia—2. A Guernsey heifer and a Friesian x Shorthorn heifer.

Anchylosis—2. Shorthorn heifers.

Gross hydrops of the foetus and membranes—4. Ayshire 2 (cow and heifer) ; Friesian 2 (heifers).

Incomplete dilatation of the cervix. This group comprised 10 cases (22 per cent). The breeds involved were Friesian—7 (5 cows, 2 heifers) ; Shorthorn—2 (cows) ; Ayrshire—1 (cow). In two the condition was further complicated by torsion, in one by twins and in another by lateral deviation of the head.

Uterine torsion. The number of a cases in this group was seven (15 per cent). They comprised Friesian—6 (5 cows, 1 heifer) ; Ayrshire—1 (heifer).

Retained foetal mummy. There were two cases, an Ayrshire cow and heifer.

THE FOETAL MEMBRANES

In the 17 cases in which the calf was alive at the time of operation the placenta was firmly adherent and no attempt was made to detach the allantois chorion. In 13 of them the membranes were expelled naturally in the normal period expected for expulsion. In those

cases under continuous observation it occurred in from 3 to 7 hours after operation but in many, as operation was performed during the evening or at night, the exact time was unknown but they were expelled by the following morning. Of the four remaining cases, in one (36) the mother died from haemorrhage soon after operation, in another (41) the mother died 3½ days later with the membranes still retained but a post-mortem examination was not carried out, while in the remaining two (1 and 3) the mothers survived but the membranes were retained 4 and 11 days respectively.

Of the 24 delayed cases in which the calf was dead at the time of operation, in 16 the allantois chorion was easily detached and removed at operation. In 11 (69 per cent) of this latter group the mother recovered (including case 38 which was destroyed 64 days after operation because of hernia). Of the eight in which it was not possible to remove the placenta at operation, four mothers recovered; one (15) expelled the membranes 2½ hours after operation, while the other three (17, 25, 26) retained them 13, 3 and 17 days respectively. In the four fatal cases they were retained until death, a period varying from 30 hours to 8 days.

CAUSES OF MATERNAL DEATHS

In 17 cases the mother died (38 per cent). The causes of death were as follows :

Shock, toxaemia and haemorrhage (acting singly or together). Six deaths occurred in a period up to 24 hours following operation. Two (44 and 36) were good risks. In the first, death was due directly to shock consequent on the severe intra-abdominal manipulation necessitated by the operation, the case being one of 180° torsion of the uterus in which it was impossible to bring the uterus adjacent to the abdominal wall. The other was a case of fatal haemorrhage from the uterine incision. Three were bad risks (29, 32 and 40). They were delayed cases in which the animal was in a state of exhaustion and toxaemia at the time of operation and was unable to withstand the superimposed shock of an abdominal interference. The final case (39), which was exceptional, was an Ayrshire cow with gross so called *Hydrops amnii* in which there was an enormous quantity of fluid in the uterus. Operation was a preparturient one but the cow was unable to withstand the sudden release of this large volume of fluid.

Early generalised peritonitis. This condition caused five deaths all being in delayed cases. In one, the operation was performed about 12 hours after the onset of second stage labour while in the remainder the delay was of 24 hours duration or longer. In one, the foetal membranes were removed at operation and in the remaining four they were retained until death. One (35) had been subjected to prolonged vaginal interference and embryotomy before admission for operation and shock and exhaustion were added factors. Death

occurred 40 hours after operation in the last case and after a period varying from 4 to 8 days in the remainder. A constant feature of these cases was a lack of uterine involution. At post-mortem examination the organ comprised a flaccid sac occupied by foetid fluid and disintegrating membranes ; in addition there was necrosis of the uterine wall around the incision. In one case (41) which died $3\frac{1}{2}$ days after operation, no post-mortem examination was performed, but it is suspected that generalised peritonitis was the cause.

Anaesthetic intoxication. One loss occurred from anaesthetic intoxication (42). The subject was an exceptionally exciteable Shorthorn cow in which 50 g. of chloral hydrate gave inadequate narcosis. It was augmented by chloroform by inhalation using a Cox type mask and allowing a good air supply. Three applications of chloroform were made over a period of five minutes and comprising in all 2 fluid ounces. The animal passed quickly from the stage of narcotic excitement to that of bulbar paralysis. This case served to emphasise the ever present dangers when using chloroform.

Delayed deaths. In two cases (37 and 38) the animals recovered from the operation but the wound of the abdominal musculature broke down and a gross subcutaneous hernia developed. They were destroyed respectively 21 and 64 days later. A third case (43) had a stormy post-operative period due to excessive peritoneal interference and subsequent wound sepsis. When discharged from hospital on the 33rd day the skin wound had healed and it was thought that all was well. It was subsequently learned that the cow died after returning home from peritonitis by extension from a wound abscess.

Operation abandoned. In one delayed case (45) of *Schistosomus reflexus* in which three of the limbs were anchylosed and impacted in the maternal pelvis, the operation was abandoned, as the foetus could not be removed from the uterus. The cow was destroyed.

DETAILS OF OPERATION

Anaesthesia. In all cases anaesthesia was first induced by the intravenous injection of chloral hydrate augmented by local infiltration of the site of incision. The dose of chloral hydrate ranged from 30 to 60 g. In assessing it, allowance was made for the animal's weight and state of health. In the majority, the quantity given to cows of average size was 50 g. and for most this dose combined with local anaesthesia was adequate, there being no movement during operation. In nine cases it was necessary to augment the narcosis. In seven of these chloroform by inhalation was used and in one of them, previously referred to, it proved fatal. In two, nembutal by intravenous injection was used to augment the chloral. It is proposed to refer to these in some deail for it is the writer's intention in future to use nembutal for augmentation of the chloral narcosis where necessary. The first was a fat Ayrshire weighing 11 cwt. gravid with

a resorbed mummy. Chloral hydrate (50 g.) was given which induced a state of deep narcosis. Anaesthesia was completed by the slow intravenous injection of nembutal 1·3 g. being used. The second was a big Friesian heifer weighing 10½ cwt. It had been the intention to give her 60 g. of chloral hydrate but owing to difficulties in making the intravenous injection, it is estimated that she received about 45 g. only. Nembutal was then given by slow intravenous injection until full anaesthesia was attained, 2 g. being used. In both instances perfect anaesthesia for operative interferences of about 35 minutes was obtained.

Control of haemorrhage during incision of the abdominal wall. The average length of the incision adopted was ten inches but when dealing with monsters such as *Schistosomus reflexus* or cases of anasarca its length was increased to 12 and even 14 inches. In the majority of cases the site was the right ventrolateral aspect of the abdomen parallel with and some four to six inches lateral to the mammary vein. On cutting the skin and exposing the panniculus carnosus two or three veins are seen crossing the site. These are occluded by double ligature before further cutting. The yellow tunic is incised exposing the rectus abdominis muscle beneath. The largest blood vessels encountered in incision of the abdominal wall traverse this site accompanied by lumbar nerves, while at the anterior commissure there is generally a large branch of the mammary vein. Care should be taken to control haemorrhage by double ligature of these vessels before cutting them. At this site the rectus abdominis is thin and the peritoneum lies immediately beneath it.

Manipulation of the uterus. Two layers of omentum lie immediately beneath the incision, and in fat animals, the more superficial one contains large fat deposits which may cause some confusion as to the identity of the structure. These layers are first snipped with scissors and then an aperture torn in them. It is probable that even by tearing, some small blood vessels will be ruptured. Haemorrhage from them should be controlled by transient forci-pressure. The abdomen can now be freely entered with the hand. In heifers, provided the dystocia is not associated with displacement of the uterus, the gravid cornu lies immediately beneath and close to the wound. The portion being palpated is the ventral aspect of the greater curvature, and the part of the foetus occupying it is the pelvic region in anterior presentation and the withers region in posterior. In the cow with her more voluminous abdomen it may be found that as she lies in the dorsal recumbent position with the abdominal muscles relaxed the uterus has fallen towards the flank. In this case the hand and arm should be introduced deeply into the abdomen between the uterus and the abdominal wall and the organ lifted up to the abdominal incision, to ensure that the uterine incision is made along the greater curvature and not to one side of it.

In protracted cases in which there is a complete loss of foetal fluids and the uterus has contracted firmly about the foetus, it may not be possible to bring the uterus adjacent to the abdominal incision because of its reduction in volume. In such cases, even when the uterus is raised in the manner described, the appropriate surface for cutting is still some three or four inches deep to the abdominal wound and it becomes necessary to incise the uterus blindly within the abdomen, a difficult and hazardous task.

In torsion of the uterus, particularly when the foetus is dead and there is a loss of fluids, even greater difficulties may be encountered. When the torsion is 180°, not only is the uterus a considerable distance from the abdominal wall but in addition it is the dorsal aspect of the greater curvature which is presented to the operator. Thus, not only is incision of the uterus " blind ", but in addition, contraction of the organ after the removal of the foetus tends to correct the displacement and effective suture of the dorsal surface may be difficult or even impossible. In any event prolonged and severe traction on the uterus will be necessary. In these cases operation may be a most formidable task occupying an hour or an hour and a half, necessitating the use of large quantities of anaesthetic. Very frequently the cumulative effect of these factors is the death of the patient from shock some 12 to 24 hours after completion of the operation.

A small incision is made through the uterine wall and the adjacent allantois chorion with a knife, taking care that the primary cut does not involve a cotyledon, and then enlarged in the forwards direction using scissors. Having regard to the manipulation of the uterus necessary to maintain it in a position suitable for incision and the lack of space in which there is to work, it is seldom that a uterine incision longer than five inches is made, but the uterus is subse quently torn during extraction of the foetus. This tearing is uncontrollable, but it has been noticed that it does not tend to involve cotyledons, and that its general direction is longitudinal rather than transverse. The length of the ultimate aperture is generally some ten to twelve inches. On tearing the amnion a variable quantity of foetal fluid escapes; it is seldom more than a few pints, except in cases of *hydrops*. Often in protracted cases there is virtually none. The fluid present may be clear and clean but it may be sanguineous and foetid. It should be stated that in adopting the site of abdominal incision described, it is unlikely that small intestine will be seen or handled during operation other than in uterine displacement cases.

In regard to removal of the foetus from the uterus it is important that its disposition shall be considered. In order that the calf shall offer the least obstruction to withdrawal, careful and precise manipulation of its limbs must be made, for in many cases its removal through the adventitious aperture in the abdominal wall will still be a " tight fit ". Moreover, care must be taken when extending

the limbs that a digit does not injure the uterine wall at some point distant from the incision; in fact, the principles which govern manipulation of foetal limbs when assisting a dystocia case per vaginam apply with no less force in caesarian section. In cases of posterior presentation the presence of the head increases the difficulties of drawing the foetal extremity into the incision. If both fore limbs are first drawn out there may be insufficient space to bring up the head with the hand, while if the head is first withdrawn difficulty is experienced in manipulating the limbs. Thus, in these cases traction cords are used. A snare is applied to the head in the orthodox manner while it is still in the uterus. Each limb is then drawn up until its digit is in the incision and snares are applied. The head is pulled out of the incision first while the limbs are extended and drawn out alternately. Traction is then applied to the whole calf, its direction being a continuation of the greater curvature of the cornu. Cases are encountered in which it is necessary to perform intra-uterine embryotomy before it is possible to extract the calf. In calves with anasarca it has been necessary to eviscerate the abdomen and thorax before the calf could be removed. In cases of *Schistosomus reflexus* it has been our practice to remove the distorted monster without resorting to intra-uterine embryotomy for such would be difficult and prolonged. Vigorous traction is applied to the extremities or to the curvature of the trunk (according to the presentation) while altering the direction of traction as required to ensure that the foetus offers the least possible obstruction to its withdrawal through the wound. It is, of course, necessary that the abdominal incision shall be adequate for the mass to be drawn through it and our experience of such cases has been that with vigorous traction a twelve to fourteen inch incision is generally adequate.

It must be recognised that in cases such as these there is considerable peritoneal trauma and this materially increases the hazards of operation. Nevertheless, provided the case is not a grossly delayed one, the mother is in good condition and the contents of the uterus are reasonably healthy, the cow is able to withstand a surprising amount of peritoneal trauma. These points are exemplified by four of the six cases of *Schistosomus reflexus* in this series in which the mother recovered without serious post-operative complications. Nevertheless, in a delayed case in which the mother is already toxaemic and the foetus and foetal membranes are putrefying, gross peritoneal trauma may be the factor which tips the scales to the side of death.

Treatment of the abdominal wound at operation and its closure. Considerable attention has been paid to the most appropriate method of dealing with the abdominal wound, firstly to reduce to the minimum the risk of post-operative hernia which is inseparable from ventral abdominal incisions and secondly to overcome the development

of pyogenic infection of the wound. Two layers of sutures are used. The deeper layer, comprising double strands of No. 4, 21 day chromic catgut, includes the abdominal tunic, the rectus abdominis and peritoneum on each side. Care must be taken that the peritoneum is included for it tends to be drawn away from the lips of the wound. The sutures are interrupted; they are placed about three-quarters of an inch apart and they include about half an inch of the structures on either side of the incision. Great care is taken to ensure that the commissures are effectively closed. Using two strands of catgut it may be found difficult effectively to draw the suture tight if the usual double twist of the "surgeon's knot" is used and for this reason a single twist is adopted for the first knot but to prevent it loosening while the reef knot is being completed, it is gripped with forceps until completion of the knot. The wound is now treated with penicillin ointment (the tubes prepared for bovine mammary injection are used). A little is squeezed into the muscle apertures between the sutures. Following this that part of the wound which is still exposed—the surface of the abdominal tunic and the lips of the panniculus carnosus—are freely treated. The superficial layer of sutures includes the skin and panniculus on each side. The sutures of fine silk (No. 4) are interrupted and again placed about three-quarters of an inch apart taking in about half an inch of tissue on each side. Care is taken not to draw them together too tightly but simply to bring the edges into comfortable apposition. These sutures are removed on the eighth day. The writer believes that the tendency to post-operative wound infection has been greatly reduced by the use of penicillin. There has been no case of severe infection since adopting this method, and in several cases healing by primary union without even superficial suture sepsis has occurred.

THE POST-OPERATIVE COURSE

In general, the post-operative course fell into three main types: those cases in which recovery was uneventful, those in which it was stormy but the animal recovered, and those in which the cow died.

Recovery uneventful. This group comprised twenty animals, 70 per cent of the total recoveries: 14 were first-calf heifers and six were cows. In 11 the calves were alive at the time of operation, in 7 they were dead while two were sterile resorbed foetuses. In ten in which the calves were living, and one in which it was dead, the foetal membranes were expelled naturally during the 24 hours succeeding operation, and in five in which they were dead (resorbed mummies excepted) they were removed during operation. In two cases they were retained three and four days respectively; one living calf and one of dead twins.

Appetite commenced on the first or second days after operation and gradually increased to normal by the 5th or 6th day. In some cases appetite was but little impaired throughout. Milk yield progressively increased. Many of these cases received no post-operative treatment, while some were given penicillin during the first few days. In some, healing of the wound was by primary union; others developed a mild degree of suture sepsis, but in none was there serious necrosis. In one a small hernia developed due to partial breakdown of the muscle wound but the skin healed by primary union.

Body temperature was very little indication as to the course of a case. It will be seen (Charts 1 to 5 inclusive) that the temperatures of animals making a good recovery fluctuated between 101° and 106°F., the majority being in the 102° to 104° range. The evidence of these cases suggests that a rise in body temperature of 2° to 3° during the early post-operative period may be regarded as a salutary sign for it will be noted later that in fatal cases body temperature tended to remain normal or rather lower than normal.

Pulse rates were also irregular but in general afforded a better indication of the animal's health than did its temperature. In most cases they were in the 90 to 110 range for the first few days after operation after which they tended, in animals making a good recovery, to fall within the 70 to 90 range. A pulse rate which persisted at over 90, and particularly at above 100, after the fourth day, was regarded with alarm and it was generally linked with advancing peritonitis.

CHART 1.

No. 1. AYRSHIRE COW. INCOMPLETE DILATATION. CALF ALIVE. MEMBRANES RETAINED 4 DAYS. RECOVERED.

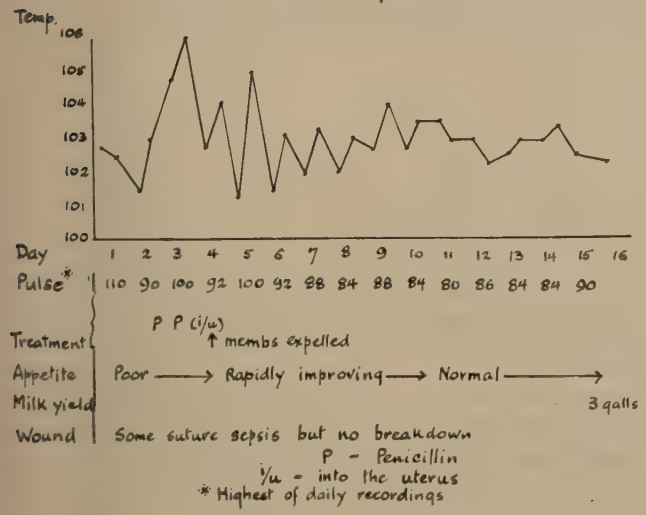
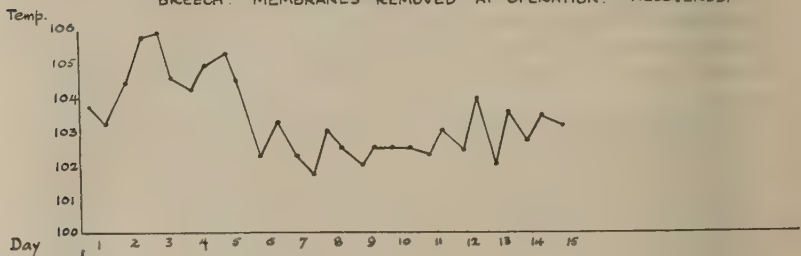


CHART 2

No 8. SHORTHORN HEIFER. CALF DEAD, ANCHYLOSIS AND BREECH. MEMBRANES REMOVED AT OPERATION. RECOVERED.

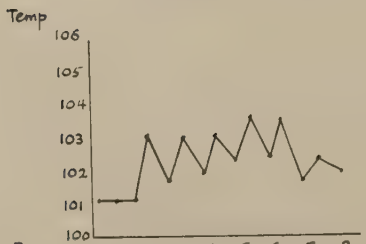


Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pulse	88 PP	88 PP	96	88	84 PP	102 (i/m)	86	90	88	96	84	82	80	80	78
Treatment	G	G	G			(i/v)									
Appetite	Slight only → Progressing to normal →														
Milk yield					11. lbs			15.lbs			22.lbs				23½ lbs
Wound	No serious check in healing														

G = glucose

CHART 3

No. 11. FRIESIAN COW. TORSION. CALF ALIVE. MEMBRANES EXPELLED NATURALLY. RECOVERED.

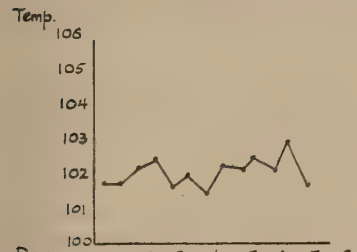


Day	1	2	3	4	5	6	7	8
Pulse	76	80	80	70	90	80	78	76

Treatment None
Milk yield Calf allowed to take all.
Wound Slight suture sepsis only
Appetite. Good throughout

CHART 4

No. 15. FRIESIAN HEIFER. OVERSIZE. CALF DEAD. MEMBRANES EXPELLED NATURALLY. RECOVERED.

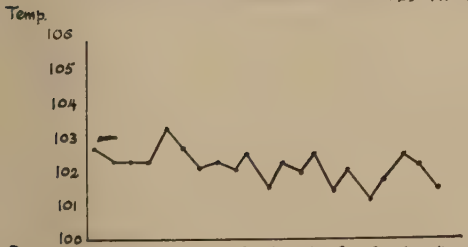


Day	1	2	3	4	5	6	7	8
Pulse	112	128	100	100	94	100	90	
Treatment { P (i/m)								
Appetite	Slight→	Good——→						
Milk yield			15 lbs			20 lbs		
Wound	Skin healed by primary union, small hernia							

(Despite rapid pulse, bright & well.)

CHART 5

No. 28. SHORTHORN HEIFER. DEAD SCHISTOSOMUS REFLEXUS. MEMBRANES REMOVED AT OPERATION. RECOVERED.



Day	1	2	3	4	5	6	7	8	9	10	11
Pulse	100	120	98	84	82	80	80	86	88	94	92
Treatment { P (i/m)											
Appetite	Good throughout										
Milk yield.			23. lbs			26. lbs				32. lbs	
Wound	Healed by primary union.										

The duration of operation and the degree of peritoneal trauma to which the animal was subjected varied greatly in members of this group. In twelve, operation was relatively straightforward and the degree of trauma represented about the normal to be expected in an operation of this magnitude, the time from incision to final suture being from 25 to 40 minutes. In eight an excessive degree of trauma was necessary. They were cases of monster formation, gross oversize with loss of fluids, and twins; in these operation time was up to one hour.

Recovery stormy. (Charts 6 to 10 inclusive). This group comprised four cows and three heifers. In three the calf was living. In one of these (20) there was no explanation for the stormy convalescence for operation was performed quickly and without excessive trauma and the membranes were naturally expelled. In another (16) excessive peritoneal trauma was the probable cause, the case being one of *Schistosomus reflexus*, while in the third (3), uterine involution was delayed and the membranes retained for eleven days. In the four in which the calf was dead (17, 24, 26, 27), three were of long delay and in two the membranes were retained respectively 13 and 17 days.

In some cases, inappetance which was the most certain indication that progress was unsatisfactory was a feature of the early post-operative period, particularly during the first four or five days. It is probable that shock played an important part for this was the group in which the membranes had been expelled or removed, but in which there was prolonged peritoneal interference. Pulse rates tended to remain high. In others appetite and pulse rate were

CHART 6

No. 13. FRIESIAN HEIFER. TORSION. CALF DEAD (putrid) MEMBRANES REMOVED AT OPERATION. RECOVERED.

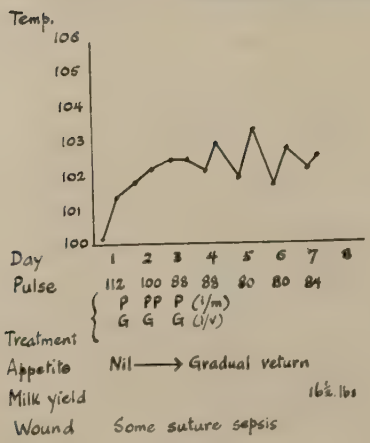
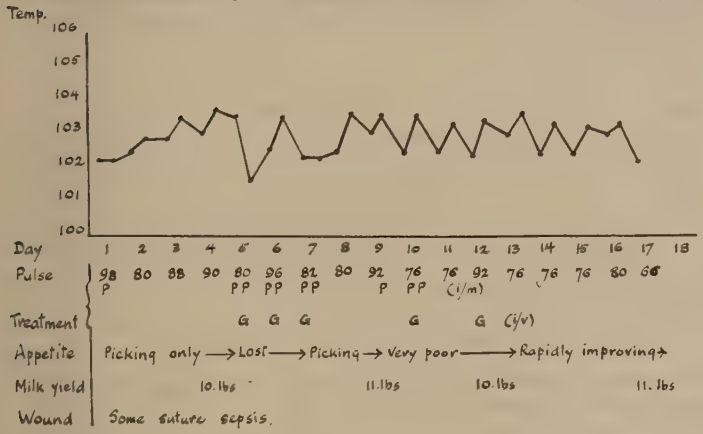


CHART 7

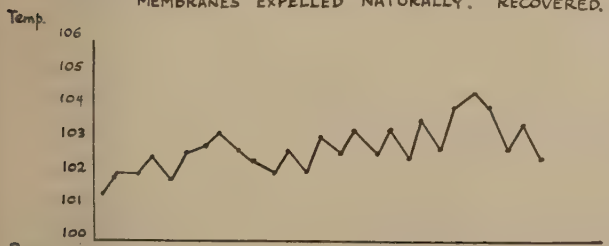
No. 16. FRIESIAN x SHORTHORN HEIFER. A LIVING SCHISTOSOMUS REFLEXUS. MEMBRANES EXPELLED NATURALLY. RECOVERED.



Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Pulse	98 P	80	88	90	80 PP	96 PP	82 PP	80	92 P	76 PP	76 (i/m)	92	76	76	76	80	66	
Treatment					G	G	G			G		G	(i/v)					
Appetite	Picking only → Lost → Picking → Very poor → Rapidly improving →																	
Milk yield					10.1bs				11.1bs			10.1bs					11. lbs	
Wound	Some suture sepsis.																	

CHART 8

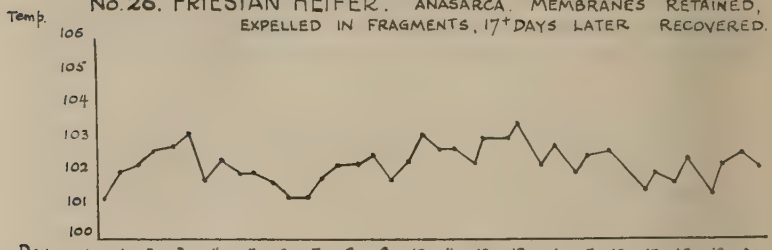
No. 20. FRIESIAN HEIFER. INCOMPLETE DILATATION. CALF ALIVE. MEMBRANES EXPELLED NATURALLY. RECOVERED.



Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pulse	96	84 G	82 G	100 G P	96 G (i/v) (i/u)	80 (i/v)	80	68	62	70	76	60	64	72	
Treatment					PP	PP	PP	(i/m)							
Appetite	Nil → Poor - variable → Fair →														
Milk yield									8.1bs					16. lbs	
Wound	Suture sepsis. Partial breakdown of muscle with hernia														

CHART 9

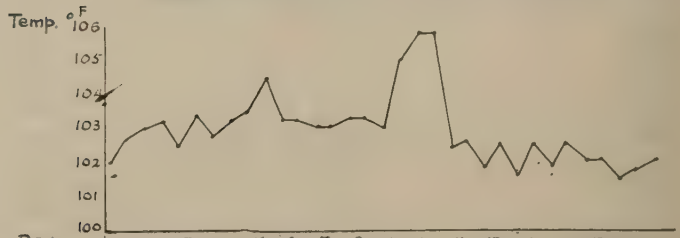
No. 26. FRIESIAN HEIFER. ANASARCA. MEMBRANES RETAINED, EXPELLED IN FRAGMENTS, 17+ DAYS LATER. RECOVERED.



Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Pulse	100	90	102	100	94	86	80	90	88	84	92	92	96	90	96	90	88	92	96	86
Treatment	PP P	PP P	PP P	PP P	(1/m) P	P	(1/u)													
Appetite	Picking only → Steadily increasing → Normal →																			
Milk yield					9. lbs			15. lbs			19 lbs			21. lbs.						25. lbs
Wound	Healed by primary union but for slight suture abscesses																			

CHART 10

No 27. FRIESIAN HEIFER - OVERSIZE, MEMBRANES REMOVED AT OPERATION. RECOVERED.



Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Pulse	86	92	90	88	94	90	84	86	86	82	74	76	80	84	76	80	80
Treatment	P				P					PP PP	PP PP	P P	(1/m) (1/u)				
Appetite	Excellent → Slightly reduced → Normal →																
Milk yield			31 lbs		34 lbs		31 lbs			28 lbs			34 lbs				40 lbs
Wound	Healed by primary union																

satisfactory during the first three or four days and then appetite became lost and the pulse rate raised. These were generally cases of retention of the foetal membranes, and it was thought that toxaemia and peritonitis were the causes of this delayed illness. In both groups penicillin was given by subcutaneous injection in doses of two to four million units per day, and in those in which the membranes were retained it was introduced into the uterus daily while inappetance persisted. In all cases of animals not eating, glucose (90 g.) was given intravenously once or twice daily.

It is difficult to assess whether the antibiotic treatment significantly influenced the course of these cases for similar ones were observed in a previously recorded group dealt with before penicillin became available for animal treatment.

Maternal death. Seven cases are reviewed under this heading. Those animals which died during operation or during the 24 hours succeeding it are excluded as also are those which were destroyed on account of hernia after apparent recovery from operation.

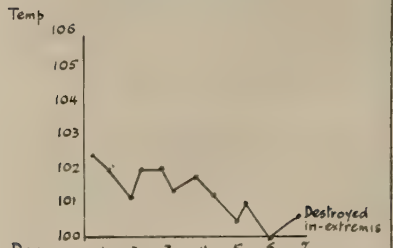
Six of them (30, 31, 33, 34, 35 and 41), died in periods varying from 40 hours to 8 days after operation. In the five in which a post mortem examination was performed, death was due to acute generalised peritonitis and it was probable also that this was the cause in the sixth. In five the calf was dead at the time of operation and in five also, the membranes were retained until death. In four, an exceptional degree of intra-abdominal interference was necessary (*Schistosomus reflexus*, twins, gross anasarca necessitating intra-uterine embryotomy, torsion).

In this group, appetite was either entirely absent throughout, or very fickle. In no instance was there a significant rise in temperature. It generally ranged between 101° and 102·5°; sometimes it was below 101°, the lowest recorded being 99·5°. With few exceptions the pulse rate fluctuated around 100 throughout (Charts 11 and 12). The most certain indications of the onset of fatal peritonitis were the development of rapid and shallow respirations the animal often emitting a short grunt with each expiration, together with marked tensing of the abdominal muscles when interfered with, and persistent recumbency. In all these cases penicillin was used freely.

The seventh case (43) was an exceptional one. After a stormy post-operative course, it was returned home on the 33rd day in the belief it was well on the way to recovery. A report was subsequently received that it died later due to peritonitis arising from an abscess in the depth of the wound.

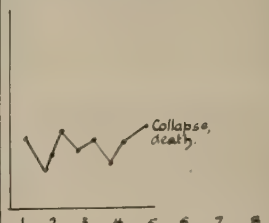
CHART 11

No 31. FRIESIAN COW. CALF DEAD. TORSION. MEMBRANES RETAINED. DIED.



Day	1	2	3	4	5	6	7
Pulse	120	90	72	92	78	102	92
Treatment (i/m)	PP	PP	PP	P		PP	(i/m)
Treatment	G	G	G	G	G	G	(i/v)
Appetite	Nil → Picking → Nil						

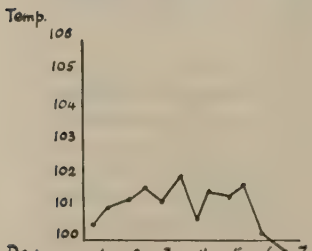
No 33. SHORTHORN COW. DEAD TWINS INCOMPLETE DILATATION MEMBRANES RETAINED. DIED.



	1	2	3	4	5	6	7	8
	116	96	100	96	108			
	PP	PP	PP	PP	(i/m)			
	P	P	P	P	(i/u)			
	G	G	G	G	(i/v)			
Appetite	Nil →							

CHART 12

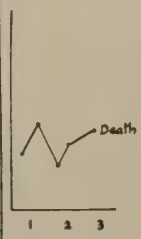
No 36. FRIESIAN x SHORTHORN HEIFER OVERSIZE. CALF DEAD. MEMBRANES RETAINED. DIED.



Day	1	2	3	4	5	6	7
Pulse	100	90	100	110	104	118	
	PP	PP	PP	PP	PP	PP	(i/m)
	S	S	S	S			(i/u)
Treatment	G	G	G	G	G		(i/v)
Appetite	Picking → Nil →						

S = streptomycin

No. 41. FRIESIAN HEIFER. OVERSIZE CALF ALIVE. MEMBRANES RETAINED. DIED.



	1	2	3
	100	100	112
	P	PP	(i/m + i/u)
	G	G	G (i/v)
	Nil →		

INFLUENCE OF OPERATION ON SUBSEQUENT BREEDING

It has, unfortunately, been impossible to obtain the subsequent breeding records of many of the animals referred to in this report but of eleven that are available, in six (4, 5, 8, 18, 19 and 21) the animals have had one or more calves since operation, while five (3, 6, 16, 20 and 24) have failed to conceive to frequent service. It will be noted that all the members of the first group made an uninterrupted recovery from operation, whereas in the second group convalescence was stormy. In those members of the second group in which it has been possible to re-examine the animal widespread adhesions of the uterus and ovaries to other viscera and to the abdominal wall have been found. It is evident that in cases in which the convalescence was stormy, extensive peritonitis had been present.

DISCUSSION

There can be no doubt that the operation of caesarian section now has an established place in bovine obstetrics. It is clear that by far the most important single factor in regard to the successful outcome of operation is the time after the onset of the second stage of labour at which it is performed. The records reported here indicate that provided the calf if alive at the time of operation, foetal fluids are still present and the uterine contents are healthy, a maternal recovery rate of not less than 75 per cent may be expected, while it is highly probable that the calf will survive the operation also. Nevertheless, even in early cases factors may exist which give rise to a fatal result. The chief of these are conditions which necessitate excessive intra-abdominal manipulation such as monster formation, gross oedema of the foetus and uterine torsion.

Prognosis is considerably influenced by the foetal membranes. In recent cases in which the calf is living it is unlikely that these can be removed at operation, but it is probable that they will be expelled naturally soon after operation. In many of the delayed cases in which the calf is dead considerable separation of the placenta has already occurred at the time of operation and in these gentle traction serves to detach and remove them. This greatly improves the prognosis. But delayed cases are encountered in which it is impossible to detach the membranes at operation, and this factor together with the delay in involution which is an almost invariable feature of delayed cases, greatly increases the incidence of maternal deaths.

In protracted cases the degree of success falls abruptly. In those in which the delay after the onset of the second stage of labour is of 36 hours or longer, the case is almost hopeless from the outset, consequent on the summative effects of physical exhaustion, uterine

inertia and toxaemia. Nevertheless, an occasional maternal recovery may occur after gross delay. The majority of cases reviewed here were of medium delay, 12 to 24 hours. In these a maternal recovery rate of 50 per cent may be expected.

It is clear that the operation *per se* is no bar to subsequent conception and normal parturition. Nevertheless it is evident that for this to occur it is necessary that recovery from operation shall be uninterrupted. It may be taken as unlikely that those animals in which the post-operative period is stormy will conceive again. But taking the overall picture as it is seen in a hospital such as this, it is likely that 50 per cent of the recovery cases will remain fertile.

It is clear that of the three breeds of cattle with which this area is chiefly populated, Friesian, Ayrshire and Shorthorn, the first is the one in which operation is most often required.

Post-operative hernia is a risk which must always be considered in relation to this operation. In a long experience of bovine abdominal surgery, the writer is convinced that the most important factor in the rapid and uncomplicated healing of surgical wounds is that the animal shall eat vigorously during the early post-operative period, and that in cases in which inappetance is a feature of the early convalescent period, the risk of breakdown of the muscle wound is greatly increased.

SUMMARY

Forty-five cases of bovine caesarian section are described in which the maternal recovery rate was 62 per cent. Among the points discussed, are the indications for operation and the breeds of cattle involved; details of anaesthesia and operative technique; factors influencing the prognosis; the post-operative course and the influence of the operation on subsequent breeding.

ACKNOWLEDGMENTS

The cases recorded in this communication represent the work and experience of the Clinical Department at the Field Station of the School and the writer must gratefully acknowledge the active part played by his present and former colleagues, particularly Messrs W. J. Jordan, W. R. Kelly and M. H. Cross.

REFERENCE

Wright, J. G. (1949). *Proc. 14th Int. vet. Cong. London*, **3**, 257.

Fig. 1

CASE No. 23. SHORTHORN HEIFER (Showing site and length of incision).

Fig. 2

CASE No. 23. SCHISTOSOMUS REFLEXUS (further abnormality : a fifth limb).

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APPENDIX

Case Protocols

1. (771) 11/3/49. Attested Ayshire cow ; 4th calf. Failure of the cervix completely to dilate. Calf alive. Amnion first seen 6 hours before operation. Chloral hydrate—50 g. intravenously plus local infiltration of line of incision. Operation uncomplicated; foetal membranes retained four days. Penicillin—1 million units introduced into the uterus on the 2nd and 3rd days. Diarrhoea commenced on the third and continued to the 6th day. Post-operative course otherwise uneventful (Chart 1). The wound generally healed well but there was some skin suture suppuration. On discharge from hospital on the 21st day, milk yield was 3 gallons per day. Subsequent history of this case unobtainable.

2. (801) 4/5/49. Non attested Friesian cow; 3rd calf. Failure of the cervix completely to dilate. Delay uncertain but calf alive. Chloral hydrate—50 g. plus local infiltration. Operation which was straightforward was performed during the evening ; the membranes were expelled during the night. Penicillin—2 million units by intramuscular injection and glucose—100 g. were given on the day following. No other treatment was given. The cow commenced to eat on the 2nd day, and from that point appetite was maintained. During the 10 days post operation temperature fluctuated between 102° and 104·7°F. being highest on the evening of the 5th day. Pulse varied between 80 and 96. The wound generally healed well but some suture suppuration occurred. Milk yield on the 16th day was 2½ gallons in addition to that taken by the calf.

3. (821) 30/5/49. Attested Friesian Cow; 3rd calf. Incomplete dilatation of the cervix. Operation performed about 10 hours after the onset of straining. Calf alive. Chloral hydrate—40 g. plus local infiltration. During operation narcosis was augmented by chloroform by inhalation. Operation was generally straightforward. An attempt was made to detach the membranes but this caused considerable bleeding from the cotyledons and was discontinued. The membranes were retained for 11 days after which they were expelled in necrotic fragments. Convalescence was stormy, particularly during the first seven days. Appetite was either absent or slight only, and there was profuse diarrhoea from the 3rd day, together with a foetid vulval discharge. 16 million units of penicillin were given by intramuscular injection and frequent intravenous injections of glucose were given. During this period temperature remained between 100·5° and 101·8° and pulse between 90 and 96. Healing of the wound was retarded, and there was considerable sloughing around the skin sutures. On the 8th day there was marked improvement and appetite became good. This persisted until the 15th day. During this period temperature ranged between 102° and 103° and pulse between 88 and 96. On the 15th day there was marked deterioration with inappetence and cold extremities; temperature fluctuating between 100 and 101·3 and pusle between 100 and 110. Glucose was given twice during the day. On the 16th day, the cow picked up again and from this point went on steadily to recover. During this period temperature remained in the 101—102 range and the pulse rate declined to 72—84. Milk yield on the 30th day was 22 lbs. in addition to that taken by the calf. Subsequently, this animal failed to conceive to repeated services.

4. (915) 17/11/49 Attested Ayrshire cow; 3rd calf. Partially resorbed foetus *in-utero*. Stilboestrol treatment had failed to cause expulsion of the calf and at the time of operation, the cervix was closed and sealed. Operation was performed 15 months after last service. Chloral hydrate 45 g. with local infiltration augmented by chloroform. The estimated age of the foetus at the time of death was 7 months (crown-rump length 20 inches). No post-operative treatment was given and recovery was uneventful. This cow has had two calves since operation.

5. (916) 18/11/49. Attested Ayrshire Heifer. Relative oversize; normal anterior presentation. Fluids lost. Delay of about 36 hours. Chloral hydrate 40 g. plus local infiltration. Operation straightforward, membranes removed. No post-operative treatment was given and recovery was uneventful. Milk yield on 18th day—24 lbs. This animal has had two calves since operation each to a single insemination and is pregnant again.

6. (917) 19/11/49. Attested Guernsey cow. Oversize with loss of fluids. Normal anterior presentation. Delay unknown; calf dead. Chloral hydrate—40 g. with local infiltration. Operation straightforward, membranes removed at the time. No post-operative treatment. Pulse rate was 100 on the first and second day after which it fluctuated between 80 and 90. Temperature varied between 101° and 103° during post-operative period. Appetite was good throughout. Milk yields on the 19th day—31 lbs. Subsequent history: an excellent recovery, a good lactation but failed to conceive again.

7. (1056) 28/4/50. Attested Ayrshire heifer. Partially resorbed foetus *in-utero*. Stilboestrol treatment failed. Operation performed 16 months after mating. Cervix closed and sealed at time of operation. Chloral hydrate—50 g., Nembutal 1·3 g. Estimated development of calf—8½ months. The umbilical cord had passed over the foetal neck. No post-operative treatment; recovery uneventful.

8. (1214) 7/10/50. Non-attested Shorthorn heifer (23 months old). Breech presentation, relative oversize, loss of fluids. Had been in labour about 24 hours; calf dead. Chloral hydrate –30 g. with local infiltration. Chloroform given during operation. Operation straightforward; membranes removed at the time. No serious check during the recovery period (Chart 2). Gave 12,819 lbs. in the lactation succeeding operation. Conceived again to artificial insemination in Oct. 1951. Has since calved.

9. (1375) 4/2/51. Non-attested Friesian cow. 2nd calf. Gross oversize (Hereford sire). Posterior presentation. Delay unknown. A dead male calf weighing 110 lbs. Chloral hydrate 45 g. with local infiltration. Chloroform during operation. Some difficulty in withdrawing head from uterus but otherwise operation was straightforward. Membranes removed at the time. Appetite maintained throughout; recovery uneventful. Milk yield when discharged from hospital on the 13th day—26 lbs.

10. (1387) 20/2/51. Non-attested Friesian heifer (dwarf). Gross oversize of calf in normal, anterior presentation. Operation performed within an hour of the onset of straining. Calf alive. Chloral hydrate 30 g., local infiltration. Operation straightforward; membranes expelled naturally 3¼ hours later. No post-operative treatment; recovery uneventful. Owner subsequently disposed of the animal.

11. (1512) 18/4/51. Non-attested Friesian cow. Torsion and incomplete dilatation. No loss of fluids, calf alive. Chloral hydrate 45 g., with local infiltration. Vulsellum forceps used for lifting uterus into position for incision. More than usual manipulation. Penicillin given on completion of operation. Membranes expelled during night. Recovery uneventful. Calf allowed to take all milk. (Chart 3).

12. (1514) 22/4/51. Non-attested Friesian heifer. Relative oversize; presentation normal. Operation performed about 4 hours after rupture of the amnion. Calf alive. Chloral hydrate 50 g., with local infiltration. Operation straightforward. Membranes expelled between 5 and 17 hours later. No post-operative treatment. Recovery uneventful. Milk yield when discharged on the 9th day—18 lbs.

13. (1561) 11/5/51. Non-attested Friesian heifer. Torsion. Animal had been in labour for longer than 24 hours. Calf dead and putrid. Chloral hydrate 50g., with local infiltration. Uterus which was very tense lifted into position for incision. Gas and foetid fluid escaped on incision of the uterus. Rather excessive uterine manipulation. Membranes removed at operation. Vigorous penicillin and glucose treament during the first three days after which there was gradual recovery (Chart 6).

14. (1573) 18/5/51. Attested Friesian heifer (20 months old). Oversize of calf, normal presentation. Delay of about 8 hours; calf alive. Chloral hydrate 30 g. with local infiltration. Operation straightforward; membranes expelled 6 hours later. Penicillin and glucose given on completion of operation, but no other treatment. Recovery uneventful. Calf allowed to take all milk.

15. (1610) 7/6/51. Non-attested Friesian heifer. Oversize; posterior presentation Delay of about 8 hours, calf dead. Chloral hydrate 40 g., with local infiltration. Operation straightforward, but presence of head increased the necessary amount of manipulation. Membranes expelled 2½ hours later. Penicillin given on completion of operation, otherwise no treatment. Recovery uneventful but for the development of a small hernia (Chart 4).

16. (1636) 24/6/51. Non-attested Friesian cross Shorthorn heifer. Schistosomus reflexus in visceral presentation. Delay of about 6 hours; foetus alive. Chloral hydrate 50 g., with local infiltration. An incision 14 inches long. Severe traction necessary to extract foetus. Penicillin given on completion. Membranes expelled 5 hours later. Convalescence stormy, particularly from the 5th to the 8th day (Chart 7).

Milk yield when discharged from hospital on the 17th day—11 lbs. Subsequently this animal failed to conceive to repeated service.

17. (1642) 1/7/51. Attested Guernsey heifer. Foetal achondroplasia and oedema. Delay of about 5 hours; calf dead. Chloral hydrate 45 g., with local infiltration. Operation straightforward. Oedematous membranes could not be detached. Heifer had a good convalescence despite retention of the membranes for 13 days. Parenteral penicillin was given for the first 5 days post-operation and intra-uterine penicillin until the 8th. Yield at the 20th day—20 lbs. This animal subsequently developed Johne's disease.

18. (1653) 9/7/51. Non-attested Shorthorn heifer. Oversize and loss of fluids. Presentation normal. Delay of about 9 hours; calf alive. Chloral hydrate 50 g., with local infiltration. Operation straightforward. Membranes expelled 3¼ hours later. No post-operative treatment; recovery uneventful. Calf allowed to take all milk. Subsequently calved normally 29/12/52.

19 (1741) 11/9/51. Non-attested Friesian cow. Schistosomus reflexus in cephalo-caudal presentation. Delay of about 12 hours; foetus alive. Chloral hydrate 50 g., with local infiltration. Incision 14 inches long. Considerable intra-uterine manipulation necessary to rotate foetus so that the head and limbs could be drawn into the wound; severe traction also necessary. Membranes expelled 7 hours later. No post-operative treatment; recovery uneventful. Yield on the 8th day—22 lbs. Conceived again without trouble and was sold just before calving.

20 (1754) 21/9/51. Attested Friesian heifer. Incomplete dilatation of the cervix. Anterior presentation, retention of a fore limb. Operation performed about 6 hours after rupture of the amnion. Calf alive. Chloral hydrate 50 g., with local infiltration. Operation straightforward; membranes expelled 4 hours later. Convalescence was, unexpectedly, very stormy (Chart 8). For 14 days after operation appetite was very poor although there were no significant variations in temperature or pulse rate. More than usual infection developed around the skin sutures and a partial breakdown of the muscle with hernia occurred. Five months after operation the animal appeared to have made a complete recovery in that her bodily condition was good and she was milking well. The hernial swelling had disappeared, but a small ring could be detected. The first observed heat occurred 4 months after operation. Examination at that time indicated uterine adhesions deep in the abdomen. She subsequently failed to conceive to repeated services.

21. (1785) 19/10/51. Attested Shorthorn heifer. Breech presentation, limb anchylosis, loss of fluids. Delay of about 12 hours, calf dead. Chloral hydrate 40 g., with local infiltration. Operation straightforward, membranes removed at the time. No post-operative treatment; recovery uneventful. Yield on discharge from hospital on the 12th day—16 lbs. This animal conceived subsequent to operation and delivered a normal heifer calf on 24/12/52.

22. (1831) 22/11/51. Attested Friesian heifer. Incomplete dilatation of the cervix; posterior presentation. Delay of about 8 hours; calf alive. Chloral hydrate 50 g., with local infiltration, augmented by chloroform. Rather more than usual manipulation owing to presence of head. No serious check in recovery but some wound sepsis developed. Calf allowed to take all milk.

23. (2016) 21/3/52. Non-attested Shorthorn heifer. Schistosomus reflexus in cephalo-caudal presentation. Delay of about 8 hours; foetus alive. Chloral hydrate 50 g., with local infiltration. Incision—12 inches. Severe manipulation required to rotate and withdraw monster. Membranes expelled 4 hours after completion. Recovery uninterrupted; yield not recorded. (Figs. 1 and 2).

24. (2031) 31/3/52. Attested Holstein-Friesian cow. Incomplete dilatation of the cervix; posterior presentation. Was known to have been in labour for 48 hours before operation. Calf dead. Chloral hydrate 50 g., with local infiltration. More than usual manipulation due to head. Membranes removed at operation. Convalescence was stormy. Vigorous antibiotic treatment; penicillin parenterally and streptomycin into the uterus. Considerable wound sepsis. Animal slowly recovered, and when discharged from hospital a month after operation, her milk yield was 3 gallons a day. This cow was seen again 7 months later. It was reported that she had milked well but had failed to conceive to frequent services. Examination revealed the uterus to be well forward in the abdomen and held by adhesions. Neither ovary could be palpated.

25. (2199) Non-attested Ayrshire heifer. Twins. Breech presentation with loss of fluids. Calves dead. Delay of 12 to 15 hours. Chloral hydrate 50 g., with local infiltration. Both foetuses occupied a single cornu. Operation straightforward but more than usual intra-uterine manipulation. Membranes retained for three days. Some suture sepsis but recovery otherwise uneventful. (Case dealt with on farm).

26. (2409) 4/10/52. Attested Friesian heifer. Anasarca. Delay of about 24 hours; calf dead. Chloral dydrate 50 g., with local infiltration. Severe traction required to extract calf (weight 96 lbs.). Membranes firmly adherent. Operation time 45 minutes. The foetal membranes were retained for 17 days after which time they were expelled in fragments. Vigorous penicillin treatment, both by intramuscular injection, and by direct introduction into the uterus, was given during the first week. The post-operative course was good (Chart 9) having regard to the prolonged retention of the membranes.

27. (2467) 4/11/52. Attested Friesian heifer. Oversize, displacement of the head, loss of fluids. Delay of about 12 hours; calf dead. Chloral hydrate 45 g., nembutal 2 g. Operation straightforward occupying about 30 minutes. Membranes removed at operation. Post-operative period uneventful but for a reduction in appetite and a rise in temperature from the 10th to the 12th day, when penicillin was given (Chart 10). The wound healed by primary union, and when discharged from hospital on the 17th day, the heifer was giving 4 gallons of milk a day.

28. (2545) 7/12/52. Non-attested Shorthorn heifer. Schistosomus reflexus in visceral presentation. Delay of about 12 hours; foetus dead. There had been extensive intra-vaginal interference, one limb having been removed by embryotomy. Chloral hydrate 50 g. with local infiltration. Incision 12 inches long. Vigorous traction necessary. Membranes removed at operation. Penicillin given on completion of operation but no further treatment. Appetite good throughout. Wound healed by primary union. Yield on discharge at 11th day—32 lbs. (Chart 5).

29. (950) 5/1/50. Non-attested Friesian cow; 3rd calf. Torsion of the uterus and oversize (calf weight 102 lbs.). Delay of at least 48 hours. Calf dead. Chloral 45 g. with local infiltration. Membranes had completely separated. Size and weight of calf necessitated severe uterine manipulation. Cow died during the night following operation from the cumulative effects of toxaemia and surgical shock.

30. (1132) 25/7/50. Non-attested Friesian Cow. *Schistosomus reflexus* in cephalo-caudal presentation. Delay of about 24 hours; calf dead. Chloral 40 g. with local infiltration. Incision 14 inches long. Monster a large one. Great difficulty experienced in rotating it and withdrawing it from the uterus. Prolonged and severe manipulation. Membranes removed at operation. Glucose given on completion and throughout the following day Cow died 40 hours after operation. Post-mortem examination showed acute generalised peritonitis. The uterus had contracted to about half its volume.

31. (1142) 3/8/50. Attested Friesian cow. Torsion and incomplete dilatation. Amnion had ruptured 48 hours before operation. Cow toxaemic. Chloral 35 g. with local infiltration. Operation not difficult, but it was impossible to remove the putrid membranes. On regaining her feet the cow commenced to strain very vigorously, and to control this 10 ml. "Parsetic" was injected epidurally. Straining ceased the following day. The cow was destroyed "in-extremis" on the 7th day. But for picking a little food on the 3rd to the 5th days, appetite was absent. Glucose was given daily by intravenous injection and penicillin was injected intramuscularly. Body temperature which was 102·5 at the time of operation remained low and during the succeeding days ranged between 99·5° and 102°. (Chart 11). Post-mortem examination showed generalised peritonitis. The uterus was flaccid and occupied by putrid membranes and fluids. The uterine wound was necrotic.

32. (1168) 5/9/50. Attested Ayrshire heifer. Torsion. Delay unknown but calf dead and membranes detached. Chloral 35 g. with local infiltration. Excessive manipulation necessary to maintain uterus in position for incision and suture. Glucose given on completion. 12 hours later the heifer was still recumbent; temperature 102·5°, pulse 120, appearance dejected. More glucose given. Death occurred about 24 hours after operation from shock. Post-mortem examination showed complete flaccidity and intense congestion of the uterus with seepage of blood from the cotyledons. The torsion was of 180 to the right (right cornu gravid), and there was great stretching of the mesovarium. Ecchymoses throughout the peritoneum. Liver pale.

33. (1182) 14/9/50. Non-attested Shorthorn cow. Incomplete dilatation of the cervix, breech presentation, loss of fluids. Delay unknown but twin heifer calves dead. Cow in fair condition. Chloral 50 g. with local infiltration. Chloroform during operation. Operation straightforward, but prolonged due to presence of twins. Both foetuses occupied the same cornu. Membranes retained. Glucose given on completion. On the day following operation the cow was eating and ruminating; temperature 101·8°. From this point there was rapid deterioration, and the animal was destroyed "in-extremis" on the 4th day. Post-mortem examination showed acute, generalised peritonitis. The uterus was flaccid and occupied by putrid membranes and fluids; the uterine incision was necrotic.

34. (1348) 17/1/51. Attested Friesian heifer. Oversize; normal, anterior presentation. Delay of about 8 hours. Heifer had been subjected to prolonged vaginal manipulation and embryotomy. Anterior half of calf's body had been removed. On admission heifer shocked and exhausted; recumbent and refusal to rise. Chloral 40 g. with local infiltration. Operation straightforward, but membranes retained. Glucose and penicillin given on completion. On the day following a little hay was eaten; temperature 101, pulse 118. From this point the animal deteriorated and despite vigorous treatment died on the 4th day. Post-mortem examination showed generalised peritonitis; uterus flaccid and occupied by putrid membranes.

35 (1505) 14/4/51. Non-attested Friesian cross heifer. Achondroplasia, oversize, breech, loss of fluids. Delay unknown but more than 24 hours. Calf dead (weight 90 lbs.). Chloral 45 g. with local infiltration. Operation straightforward; membranes retained. Despite vigorous treatment the animal died on the 7th day from generalised peritonitis (Chart 12).

36. (1515) 21/4/51. A big fat Shorthorn cow; non-attested. Incomplete dilatation of the cervix and lateral deviation of the head. An early case, calf alive but fluids largely expelled. Chloral hydrate 50 g. with chloroform. Left ventral incision used. Great difficulty in bringing the uterus adjacent to the abdominal wall for incision. After withdrawal of the calf, uterine contractions caused the uterus to be drawn deeply into the abdomen and it was impossible to complete the suturing of the organ. 12 hours after operation the cow was still recumbent with a pulse of 70 and a temperature of 101·4°; respirations 24. At 18 hours, pulse 120, temperature 100·6°, respirations shallow and rapid. Glucose and normal saline given intravenously. Stilboestrol 15 mg. injected intramuscularly followed half an hour later by pituitary extract 5 ml. At 20 hours, pulse 128, temperature 99·2°, respirations 52. Animal destroyed "in-extremis" at 22 hours. Port-mortem examination—peritoneal cavity contained many gallons of fluid, chiefly blood due to haemorrhage from the uterine incision. Muscles had a "bled out" appearance.

37. (1611) 7/6/51. Non-attested Friesian heifer. Animal very undersized. Oversize of the foetus and loss of fluids. Delay of about 8 hours, calf alive. Chloral 35 g. with local infiltration. Operation straightforward; membranes expelled during night following operation. On the following day swelling of the region of the wound occurred. At first this was thought to be a haematoma but it was subsequently found to be a hernia. During the first three days the heifer was very well; appetite was good and pulse rate satisfactory (74—100). From the 4th to the 8th day the heifer showed abdominal pain and frequently looked around in the direction of the wound. Food was refused. In the light of subsequent events it is thought that there was partial strangulation of the hernia during this period. From this point pain subsided, the heifer became bright and appetite returned. The calf was allowed to take all the milk and it thrived. On the 11th day it was decided to explore the swelling. Chloral hydrate was given slowly into the jugular vein and at 3½ minutes after 20 g. had been injected light anaesthesia was present. On opening the skin wound, a small aperture was found in the musculature at both the anterior and posterior ends of the incision, and through these, small intestine had passed and underrun the skin. There were extensive adhesions of the bowel both to itself and to the subcutaneous connective tissue. Between the two apertures the muscle wound had healed. The adhesions were broken down and the herniae reduced. Finally the apertures were closed by suture. Penicillin was given. This second operation caused no malaise and on the following day the heifer was bright and eating normally. The hernia, however, recurred 3 days later and further adhesions developed. Ultimately, an intestinal fistula developed by spread of infection from a suture abscess to the bowel wall, but despite this the heifer was in relatively good health. She was destroyed on the 21st day.

38. (1649) 4/7/51. Attested Ayrshire heifer. Foetal anasarca. Delay unknown but prolonged. Placenta partially detached and hanging from vulva. Chloral 50 g. gave deep anaesthesia and some ruminal contents trickled from the nostrils. A 10 inch incision was at first employed but it later became necessary to enlarge it to 15 inches to remove the grossly enlarged and oedematous foetus. To reduce the size of the calf multiple skin incisions were made and the chest and abdomen were eviscerated. Even when this was done severe traction was necessary to withdraw it with gross tearing of the uterus. Large quantities of foetid fluid escaped into the abdomen. The foetal membranes had largely separated and were easily removed. During the first 13 days post operation the heifer was very ill; appetite was almost absent, and milk secretion was negligible. Temperature occasionally reached 104, but generally, was about 102. The pulse was always fast, ranging from 96 to 104. During this period, 22 million units of penicillin were given intramuscularly, and glucose was injected daily. By the 10th day extensive wound sepsis and necrosis were apparent and there was considerable local swelling. From the 14th day there was great improvement; appetite steadily increased as did milk yield (21st day—10½ lbs, 35th day—16 lbs., 50th day—20½ lbs.). Pulse rate fell to the 66—80 range, and the wound slowly healed by granulation. It became apparent that, in addition, the muscle wound had broken down and that the swelling was due to hernia. The heifer was destroyed in the belief that she had ceased to be economically useful.

39. (1658) 23/7/51. Attested Ayrshire cow, 4th pregnancy. Animal stated to be about 6 months pregnant. Enormous distension of the abdomen due to hydrops amnii. Animal recumbent and unable to rise, although it made frequent attempts to do so. Respirations distressed. Pulse 76, temperature 101. (An attempt, 8 days previously, to provoke expulsion of the uterine contents by the action of stilboestrol, 30 mg. by intramuscular injection, had failed). Chloral hydrate 50 g. with local infiltration. The uterus was tightly pressed against the abdominal wall, and on incision of the adjacent cornu, a jet of fluid, 2 inches in diameter, gushed out under great pressure. It is estimated that some 50 gallons of fluid escaped. A foetus of normal appearance and about 6 months development was removed. By this time the uterus had largely collapsed and no other foetus was palpated. Operation was completed and 20 ml. "Histantin" injected. Four hours later the cow rose to her feet. There were muscle tremors; pulse 76, respirations 44. At 8 hours pulse and respirations were unchanged and a further injection of "Histantin" was given. The cow died during the night. Post-mortem examination showed the uterus to be greatly enlarged and almost filling the abdominal cavity; it still contained a large volume of fluid. In the cornu distal to the incision a second foetus was found. This was a grossly oedematous monster.

40. (1739) 10/9/51. Attested Friesian heifer. Delay unknown; calf dead. Oversize, posterior presentation, loss of fluids. The hind limbs were protruding about 6 inches from the vulva. Chloral hydrate 45 g. with local infiltration. Left ventral incision. The uterus could not be raised to the abdominal wall and it was necessary to incise it within the abdomen. Owing to the contraction of the uterus on the foetus it was found impossible to draw the head and fore limbs through the incision. In order to extract the foetus, it was necessary to push the hind parts of the foetus forwards through the vagina into the uterus, then rotate the foetus within the uterus, and finally extract it via the incision by the hind limbs. This necessitated gross intra-abdominal interference. On completion 120 g. glucose in a litre of normal saline was injected intravenously. The heifer died 6 hours later. She regained consciousness but did not rise.

41. (1748) 17/9/51. Attested Friesian heifer. Said by owner to have been calving for two days. Incomplete dilatation of the cervix, vulva very small. Calf in anterior presentation; alive when taken from the uterus but died shortly afterwards. Chloral hydrate 50 g. with local infiltration. Operation straightforward and quickly performed. Membranes retained. This heifer died 3½ days later. There was inappetence throughout; pulse fluctuated between 100 and 120 and temperature between 101·8° and 102·4°. Penicillin and glucose treatment was given. The fatal termination in this case was unexpected and unexplained. A post-mortem examination was not performed.

42. (1988) 8/3/52. Attested Shorthorn cow. Said to have been in labour all the previous night. Operation performed at noon. Oversize in anterior presentation; calf dead. The cow was very exciteable. Pulse rate prior to operation 96. Animal cast with some difficulty, and 50 g. chloral hydrate injected intravenously. The site was infiltrated. Movement commenced during incision of the musculature and chloroform by th

open mask method was administered. In all 2 ozs. of chloroform were given in 5 minutes. The animal passed quickly from a stage of narcotic excitement to bulbar paralysis with respiratory failure.

43. (2046) 6/4/52. Non-attested Friesian cow. Duration of labour unknown but calf alive. Torsion of 180°. Rolling and abdominal balottement failed to reduce the torsion. Chloral hydrate 48 g. with local infiltration. No difficulty was experienced in incising the uterus but its subsequent suture was very difficult owing to the contracting organ receding into the abdomen. This necessitated excessive traction on the uterus and increased the time of operation. The membrane were expelled 7 hours later. Convalescence was stormy particularly during the first week. On no day was appetite completely lost but it was very fickle. During this period the pulse rate fluctuated between 94 and 120 and the temperature between 102° and 104°. Penicillin was intramuscularly injected twice or three times daily, and once daily into the uterus. From this point the cow slowly improved, although there was considerable wound sepsis. On discharge from hospital on the 33rd day, the cow was bright, her appetite was normal and the skin wound had healed. The calf was allowed to take all the milk. Subsequently it was learned that this cow died shortly after returning home from peritonitis due to an operation abscess.

44. (2380) 18/9/52. Attested Ayrshire heifer. Had been showing signs of calving for two days. Torsion of the uterus; two fore limbs through the cervix to the extent of 6 inches. Chloral hydrate 50 g. with local infiltration and chloroform during the later stages of operation. The torsion could not be corrected nor could the uterus be drawn to the abdominal wall. Incision of the uterus was made within the abdomen. The calf was removed with difficulty. Suture of the uterus was most difficult due to retraction of the organ into the abdomen. Manipulation greatly excessive. Operation occupied 1½ hours. Glucose in normal saline given on completion. The heifer rose to her feet one hour later; pulse 80. Penicillin and more glucose-saline given. The following morning the placenta was partly hanging from the vulva. Pulse was rapid and too weak to count. The heifer died 23 hours after operation. Post-mortem examination showed the abdominal cavity to contain blood-stained serous fluid with a thin fibrinous deposit on the organs. The uterine incision was found to be on the dorsal aspect of the gravid horn and extending almost to the cervix indicating that the torsion was one of 180°. The uterus had undergone some involution, its diameter being about 12 inches. There was seepage of blood from the cotyledons.

45. (2495) 17/11/52. Non-attested Friesian cross Shorthorn cow. Delay of about 18 hours. *Schistosomus reflexus* in cephalo-caudal presentation impacted in the pelvis. Chloral hydrate 50 g. with local infiltration. The monster was a very large one and it was impossible to remove it from the uterus. Operation was abandoned and the cow destroyed.

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RUBARTH'S DISEASE
(Canine Virus Hepatitis)

I. Reaction of Dogs to the Conjunctival Instillation of the Virus

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INTRODUCTION

Rubarth's disease or canine virus hepatitis (H.C.C.) has been diagnosed and the virus isolated by many workers in various parts of the world. The usual methods employed for the experimental production of the disease have been intraperitoneal (Rubarth, 1947; Parry and Larin, 1951) or less commonly, intra-ocular inoculation of the virus (Evans, Yamamura and Green, 1943). Both methods are satisfactory in producing severe, if not fatal, reactions. The local "eye reaction" described here is induced by the instillation of the virus into the conjunctival sac of the dog.

METHODS

The virus used was the "Bec" strain, maintained by passage in dogs and foxes. For all the experiments described in this paper a freeze-dried suspension of dog liver (D56) which had consistently proved fatal for dogs and foxes by intraperitoneal or intra-ocular inoculation was used.

The virus suspension was prepared either with or without the addition of sterile powdered glass in 1/20 to 1/100 in beef-heart infusion broth free of dextrose. Two drops were instilled in the conjunctival sac of one or both eyes. Twenty-four dogs of different breeds were used.

RESULTS

Reaction of Dogs to the Conjunctival Instillation of the Virus in combination with powdered Glass

Experiment 1. Two dogs (52 and 54) known to be immune to H.C.C. and two susceptible dogs (57 and 58) were inoculated on 21st July, 1952, each receiving the virus suspension plus powdered glass in both eyes. No thermal or clinical reaction was demonstrable until the afternoon of the fourth day after inoculation, when it was decided to give a second dose to 52 and 58. On the following day the two susceptible animals showed oedema of the conjunctiva and of the *membrana nictitans* which rapidly increased during the following

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24 hours and then tended to diminish, so that the eye appeared normal by the tenth day (five days after the initial reaction.) This will be referred to as the "eye reaction". In addition to the conjunctival oedema, a thermal reaction of two to three days duration, acute tonsilitis and the enlargement of the cervical lymph nodes and thyroid gland were observed at the same time. However, both dogs retained their appetite and appeared otherwise fit. The two immune dogs showed neither thermal nor clinical reaction.

The complement-fixation test on the sera of the four dogs before and after experimental inoculation confirmed that the clinical infection was that of H.C.C.

Experiment 2. Six dogs were used: two (54 and 55) known to be immune to H.C.C., two (57 and 58) which had recovered from experiment 1 and two susceptible (59 and 60). They were inoculated on 30th August, 1952 by the instillation of the virus plus powdered glass. The first and second groups showed neither thermal nor clinical reaction. The susceptible animals showed thermal reactions beginning in the afternoon of the fourth day and lasting for three days. On the morning of the sixth day both showed the typical "eye reaction" with acute tonsilitis and cervical lymphadenitis. Five days later, i.e. eleven days after inoculation, 59 had recovered and its eyes, tonsils and lymph nodes appeared normal. Dog 60 was destroyed on the sixth day after inoculation, in order to study the distribution of the virus-antigen in the body tissues (Table 3). It is apparent from this table that the tonsils, thyroid and thymus contained at least eight times as much virus-antigen per g. as the liver of the same animal. No virus-antigen was detectable in the eye, gall-bladder, lung, trachea, heart, pancreas, urinary bladder and testicles nor in the urine.

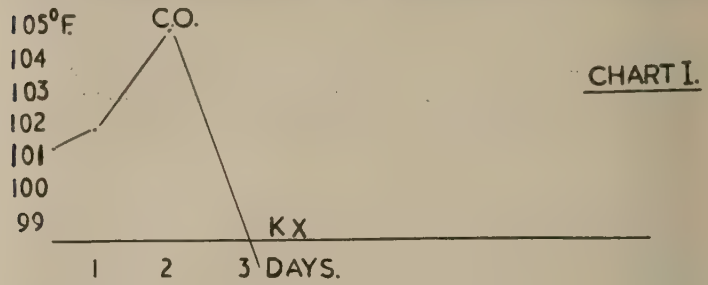
The post-mortem lesions in the dog 60 were confined to severe tonsilitis, enlargement of the thyroid to more than four times its normal size, enlargement and oedema of the thymus and an acute lymphadenitis especially marked in the cervical nodes. (Fig. 3).

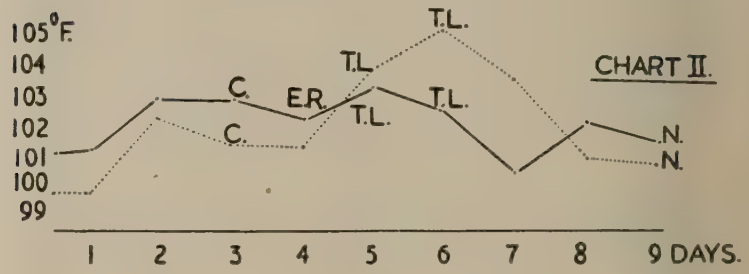
Dog 58 was killed on the sixth day and dog 55 on the 12th day for the study of the distribution of the virus-antigen in immune animals. On post-mortem examination no abnormality was noticed. In dog 58 virus-antigen was present in small amounts in the kidney, spleen, liver and cervical lymph node, while the tonsils, thyroid and thymus contained no antigen (Table 3). Tissue extracts of dog 55 did not show any traces of virus-antigen. The complement-fixation test on the serum of dog 59, before and after inoculation, confirmed the clinical evidence of H.C.C. infection.

The Effect of the Instillation of the Virus alone

Experiment 3. At this stage, it was decided to compare the effect of the instillation of the "H.C.C." virus alone and in combination with powdered glass. Three dogs were used: 54 was an

immune animal, 86 a susceptible eight months old Alsatian, and 87 a litter sister of 86. Dogs 54 and 86 received two drops of virus alone in the right eye and two drops of virus plus glass in the left eye.





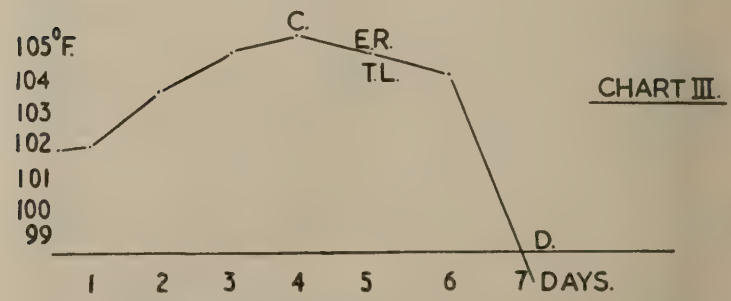


Chart I Dog 56 inoculated intra-ocularly.
KX : killed in extremis.

Chart II Dog 86 inoculated by conjunctival instillation.
Dog 87 contact; placed at time of inoculation.

Chart III Dog 85 inoculated by intraperitoneal as well as by conjunctival instillation, intranasal and intradermal routes.

C : conjunctivitis
ER : eye reaction
L : lymphadenitis
T : acute tonsilitis
C.O : corneal opacity
N : normal
D : dead

Dog 87 was not inoculated but was placed in contact at the time of inoculation. (Chart II).

Dog 54 manifested neither thermal nor clinical reactions. Dog 86 showed conjunctivitis on the third day accompanied by a slight thermal reaction. The "eye reaction" appeared in both eyes on the fourth day, became prominent during the following two days and had disappeared by the eighth day. Acute tonsilitis and lymphadenitis were noticed on the fourth, fifth and sixth day after inoculation. All symptoms had disappeared by the tenth day. Dog 87 showed conjunctivitis at the same time as 86. A definite thermal reaction was present on the fifth, sixth and seventh days. Acute tonsilitis and severe lymphadenitis were seen but the condition of the animal returned to normal by the tenth day. No "eye reaction" occurred. None of the dogs was ever off its food or clinically sick. The complement-fixation test on sera collected before and after inoculation confirmed that the infection was that of H.C.C.

All the dogs which had reacted and subsequently recovered were found to be solidly immune on challenge with at least 1000 M.L.D. of liver suspension inoculated by the intraperitoneal route.

Titration of the Virus by the Instillation Method

Experiment 4. The three dogs which were used were 88 (8 weeks old Labrador), 89 and 90 (8 weeks old Foxterriers). The route of inoculation, dilution and results are indicated in Table 1. A fourth susceptible control was placed in contact four days after the first three dogs had been inoculated. From an experiment of this nature, it was found that the "eye reaction" can be demonstrated only when lower dilutions of virus are instilled. The "eye reaction" subsided and the dogs were clinically normal by the 10th day.

Variation of the Route of Inoculation

Experiment 5. The six dogs which were used were two immune to H.C.C. (54 and 57), two susceptible Beagles (82 and 83) and two susceptible cross-bred Alsatians (84 and 85). It was decided, for comparative purposes, to include other routes of inoculation. The dogs were inoculated on 13.9.52. In the case of intranasal and intraperitoneal inoculation, virus alone was given but the conjunctival instillation comprised virus plus powdered glass in one eye.

The details are as follows: Nos. 54, 82 and 84 received an intranasal dose of 1.0 ml. and conjunctival instillation; No. 57 an intraperitoneal dose of 5.0 ml.; No. 83 an intranasal dose of 1·0 ml.; and No. 85 a combination of intranasal 1·0 ml., intraperitoneal 5·0 ml., intradermal 0·5 ml., and conjunctival instillation.

Dogs 54 and 57 showed neither thermal nor clinical reaction. Dog 82, showed a thermal and clinical reaction on the 5th day after inoculation. Acute tonsilitis, enlargement of the cervical lymph nodes and the eye reaction were observed. The condition improved by the ninth day. Dog 83 showed a thermal reaction

TABLE 1

Dog No.	Eye inoculated	Virus dilution	Clinical Remarks						Challenge				
			Conjunctivitis	"Eye reaction"	Thermal reaction	Acute tonsilitis	Lymphadenitis	Result	Days after 1st inoculation	Inoculated eye	Virus Diltution	"Eye reaction"	Result
88	Right	None	+	o	+	+	+	Recovered	17	Right	1/5	o	IMMUNE
	Left	1×10^2	+	++++									
89	Right	1×10^3	+	+++	+	+	+	,,	17	Right	1/5	o	IMMUNE
	Left	1×10^4	+	++									
90	Right	1×10^5	+	+	+	+	+	,,	17	Right	1/5	o	IMMUNE
	Left	1×10^6	+	o									
91*	Right	None	+	o	+	+	+	,,	13	Right	1/5	o	IMMUNE
	Left	None	+	o									

* — Dog 91 placed in-contact with 88, 89 and 90 four days after inoculation
ø — ++++ : Severe oedema, eyelids nearly closed
\+ : a mild reaction
o : no reaction

after 72 hrs. without any accompanying "eye reaction". Acute tonsilitis and enlarged lymph nodes were observed on the sixth day, but by the ninth day the swellings had subsided. Dog 84 showed a thermal reaction on the fourth day and the typical "eye reaction" on the fifth day. This was accompanied by acute tonsilitis and enlarged lymph nodes. The animal was killed on the sixth day for the study of the distribution of the virus-antigen in its body tissues. The changes noted on post-mortem examination were severe tonsilitis and enlarged lymph nodes, oedema of the thymus and also of the periocular tissues and enlargement of the thyroid to four times its normal size. Dog 85 showed a definite thermal reaction after 48 hours and the "eye reaction" on the fifth day. It was found dead on the seventh day. The post-mortem examination revealed a severe tonsilitis, congestion of the liver, kidney and spleen, congestion and enlargement of lymph nodes; distention of the pericardium with sanguinous fluid. Although the trachea was full of blood, the lungs appeared normal. The thymus was enlarged and oedematous and there was oedema of the periorbital tissues.

Experiment 6. A litter of seven puppies, four weeks old, bred from parents immune to H.C.C. were used in this experiment. They were inoculated while they were still on the dam, with a 1/100 liver suspension. Puppy 61 received 0·3 ml. intracerebrally; No. 62, 0·5 ml. intranasally; No. 64, 2 ml. intraperitoneally; No. 65, 2 ml. subcutaneously; No. 66, 0·2 ml. intradermally. In No. 63, two drops of a 1/20 suspension plus powdered glass was instilled in the conjunctival sac. No. C which was uninoculated but was left in contact with the others, showed no thermal or clinical reaction Nos. 62, 63 and 64 were reinoculated intraperitoneally (2 ml. of 1/100) after 10 days, without the production of any thermal or clinical reaction. No. 61 was killed on the 16th day after inoculation. The examination failed to reveal any abnormality. Tissues collected and prepared as antigens gave negative results with H.C.C. positive serum with the exception of the kidney which gave a very low titre.

The Immune Response of Dogs recovered after Instillation of the Virus in the Conjunctival-sac

It was found that dogs inoculated by this method recovered within 10 days from the time of inoculation and on challenge by reinoculation of the living virus intraperitoneally or by conjunctival instillation they were immune. With the exception of dog No. 88 which on challenge showed a slight thermal reaction only, on the whole neither thermal nor clinical reaction occur. Table 2 indicates the summary of the inoculation and the reinoculation of the dogs used in these experiments.

The Distribution of the Virus-antigen in the Body-tissue of Infected, Recovered and Immune Animals as determined by the Complement-fixation Test

Tissues collected from infected and recovered animals, killed at different periods from the time of infection, were extracted and the distribution of the virus-antigen was determined by the complement-fixation test. The results are recorded in Table 3. As a negative control the liver of a normal dog was extracted by the same method and the positive antigen was prepared from the liver of dog 56 which was killed 'in extremis' following intra-ocular inoculation. The liver of this dog yielded a good antigen (+ve titre 1/320) which has been kept freeze-dried or frozen at -20°C. from the time of preparation. The methods used in preparing antigen and in making the tests are those described by the author in a separate article to be published shortly.

It would appear that the concentration of virus-antigen in the tissues of infected animals varies considerably. For example in dog 60 the titre of the tonsil and the thyroid was 1/320, that of the mesenteric lymph node 1/10. In dog 84, however, the titre of the tonsil was 1/80, the thyroid 1/20, and the mesenteric lymph node 1/320. It would be interesting to know whether this variation was reflected in a similar variation in virus content but, unfortunately, we could not afford the animals that would be necessary for comparative titrations. No reason for this variation can be given. An immune animal killed six days after reinoculation with living virus may show some virus-antigen in the kidney, spleen, liver and a low titre 1/10 in the tonsils while an infected animal shows a higher titre 1/40 to 1/320. If an immune animal is killed 12 days or more after the last reinoculation little or no virus antigen can be demonstrated (e.g. 52, 55 and 57). However such animals show in all cases a high titre of complement fixing antibodies, 1/32 to 1/128.

DISCUSSION

Variation in the symptomatology of canine virus hepatitis, especially in the course of non-fatal infections during which there develops an immunity both against a further natural attack and against experimental challenge, has long been a subject which needed further investigation. Rubarth (1947), in his studies, used the intraperitoneal route to inoculate his experimental dogs and observed a high percentage of fatal results. His animals gave a thermal reaction within 24 to 48 hours and developed the acute or subacute form of the disease. Evans, *et al.* (1943) reported the results obtained by using the intra-ocular technique in infecting dogs, foxes and wolves. This method resulted in the production of corneal opacity, followed usually by a rapid, fatal course. Chart 1 shows the thermal reaction in one of our experimental dogs inocu-

TABLE 2

Dog No.	*Condition of animal at time of inoculation*	*Date of inoculation*	*Route of inoculation*	*Clinical Remarks*			
				Thermal reaction	*Conjunctivitis*	*Eye-reaction*	*Acute tonsilitis*
52	Immune	21. 7.52	Instillation	—	—	—	—
54	,,	21. 7.52	,,	—	—	-	—
54	,,	30. 8.52	,,	—	—	—	-
54	,,	13. 9.52	,, +I/N	—	—	-	—
54	,,	1.11.52	,,	—	—	—	—
54	,,	11.11.52	I/P	—	-	-	—
55	,,	30. 8.52	Instillation	—	—	—	—
55	,,	1.11.52	I/P	—	—	-	—
57	Susceptible	21. 7.52	Instillation	+	+	+	+
57	Recovered	30. 8.52	,,	—	—	-	—
57	Immune	13. 9.52	I/P	—	—	—	—
58	Susceptible	21. 7.52	Instillation	+	+	+	+
58	Recovered	30. 8.52	,,	—	—	—	—
59	Susceptible	30. 8.52	,,	+	+	+	+
60	Susceptible	30. 8.52	,,	+	+	+	+
61	4 weeks old	13. 9.52	I/C	—	—	—	—
62		,,	I/C	—	—	-	—
62		23. 9.52	I/P	—	—	—	—
63		13. 9.52	Instillation	—	—	—	—
63	Bred from Parents	23. 9.52	I/P	—	—	—	—
64	immune to	13. 9.52	I/P	—	—	—	—
64	H.C.C. still on the dam	23. 9.52	I/P	—	—	—	—
65		13. 9.52	S/C	—	—	—	—
66		13. 9.52	I/D	—	—	—	—
C.		13. 9.52	Contact	—	—	—	—
82	Susceptible	13. 9.52	Instillation + I/N	+	+	+	+
83	,,	13. 9.52	I/N	+	+	—	+
84	,,	13. 9.52	Instillation + I/N	+	+	+	+
85	,,	13. 9.52	Instillation & I/N, I/D & I/P	+	+	+	+
86	,,	1.11.52	Instillation	+	+	+	+
86	Recovered	11.11.52	I/P	—	—	-	—
87	Susceptible	1.11.52	Contact	+	+	—	+
87	Recovered	11.11.52	I/P	—	—	—	—
88	Susceptible	16. 1.53	Instillation	+	+	+	+
88	Recovered	2. 2.53	,,	+	—	—	—
89	Susceptible	16. 1.53	,,	+	+	+	+
89	Recovered	2. 2.53	,,	—	—	—	—
90	Susceptible	16. 1.53	,,	+	+	±	+
90	Recovered	2. 2.53	,,	—	—	—	—
91	Susceptible	20. 1.53	Contact to 88	+	+	—	+
91	Recovered	2. 2.53	Instillation	—	—	—	—

TABLE 3

Condition and tissue examined	Dog number 52	55	57	58	60	61	63	64	82	84	85
Experiment number ..	1	2	5	2	2	6	6	6	6	6	6
Condition of animal ..	Immune	Immune	Immune	Immune	Reactor	Immune	Immune	Immune	Recovering	Reactor	Dead
Days after last inoculation	7	12	54	6	6	16	78	78	16	6	7
Membrana nictitans ..	0	0	0	0	1/10	0	0	0		1/20	0
Tonsil	0	0	0	1/10	1/320	0	0	0	1/10	1/80	1/40
Thyroid	0	0	0	0	1/320	0	0	0	0	1/20	0
Thymus	0	0	0	0	1/160	0	0	0	0	0	1/10
Lymph node (cervical) ..	0	0	0	1/10	—	0	0	0	1/10	1/40	1/20
Lung	0	0	0	0	0	0	0	0	0	0	0
Liver	0	0	0	1/20	1/20	0	0	0	1/10	1/10	1/10
Spleen	0	0	0	1/40	1/20	0	0	0	1/20	1/10	0
Mesenteric Lymph node ..	0	0	0	—	1/10	0	0	0	0	1/320	1/10
Kidney	1/20	0	0	1/80	1/80	1/10	0	0	1/10	1/20	1/40
Urinary bladder	0	0	0	0	0	0	0	0	0	1/10	0
Urine	0	0	0	0	0	0	0	0	0	0	0
Brain	0	0	0	—	—	0	0	0	—	1/10	1/10

lated by this method from which material was taken as our standard H.C.C. dog liver-virus or virus-antigen referred to as D56 in the present investigation.

The object of the present investigation was to find a route by which the virus could be administered so as to produce a non-fatal infection, thus simulating the natural, inapparent infection. It appears from the experiments reported in this paper that if, instead of being inoculated intra-ocularly, the virus is instilled into the conjunctival sac of a susceptible dog, it produces what may be described as an "eye reaction". This is an oedema of the conjunctiva and the *membrane nictitans*, appearing four to six days after the virus is instilled, which tends to disperse within five days, leaving the animal solidly immune against further infection. The instillation of the Glasgow 1S23 strain of neurotropic distemper virus "DCX" (Mansi, 1951), in the conjunctival sac of susceptible dogs has not produced such a reaction.

In the experiments reported here, acute tonsilitis in which condition the tonsils appeared much enlarged, reddish pink in colour, with a distinctly granular surface was observed in all infected dogs including those who caught the infection by contact, e.g. 87 and 91, which might indicate the possible route of natural infection. This type of tonsilitis was reported by Coffin (1948) in his observations on its relation to H.C.C.

The enlargement of the lymph nodes especially the cervical lymph nodes, which appeared simultaneously with the other reactions and lasted for two to four days, was associated with the presence of virus-antigen, as indicated by the complement-fixation test on those animals killed on the sixth day after infection (Table 3). The tonsils were also a good source of virus-antigen. The virus-antigen content of other tissues varied considerably as indicated in Table 3.

The powdered glass used in this experiment was so finely ground and so small in quantity that it did not cause any non-specific inflammation or reaction in the known immune dogs. Furthermore the "eye reaction" occurred between the 4th to 6th day after instillation of the suspension, thus eliminating the possibility of traumatic injury to the tissue as an immediate cause of the reaction. The conjunctivitis was of a non-purulent type.

The important observation about this method of experimental infection is that a definite "eye reaction" will take place in susceptible dogs, while no reaction is induced in an animal immune to H.C.C. When a reaction occurs in a susceptible dog, it takes the form of a mild clinical condition without anorexia and of short duration. Immunity to further experimental infection by the intraperitoneal inoculation of at least 1000 M.L.D. will have developed ten days from the time of instillation of the virus into the conjunctival sac.

With the disappearance of the "eye reaction", the virus-antigen also disappears from the eye and the conjunctiva as well as from the lymph nodes. It is possible that at this stage the animal is no longer infective to susceptible dogs.

The fact that none of the dogs inoculated into the conjunctival sac alone developed severe illness or died, suggests that virulent material given in this way may prove a satisfactory method of immunizing dogs against H.C.C. The immunizing agent would be cheap and easy to prepare.

SUMMARY

A conjunctival reaction, termed the "eye reaction", to the virus of canine hepatitis is described and the serological and immunological findings following inoculation are discussed.

In addition to the "eye reaction", other symptoms shown are acute tonsilitis, lymphadenitis and a febrile reaction.

The clinical picture in relation to the different experimental routes of infection is illustrated in a series of charts.

It was found that puppies sucking an immune mother, were resistant to infection by various routes.

Evidence is provided that the dog can be actively immunized by instillation of the living virus in the conjunctival sac and the method seems worthy of trial under field conditions.

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REFERENCES

Coffin, D. L. (1948). *J. Amer. vet. med. Assoc.*, **112**, 355.
Evans, C. A., Yamamura, H. Y., and Green, R. G. (1943). *Science*, **98**, 45.
Mansi, W. (1951). *Brit. vet. J.*, **107**, 214.
Parry, H. B., and Larin, N. M. (1951). *Vet. Rec.*, **63**, 833.
Rubarth, S. (1947). *Acta. path. Scand. Supl.*, **69**, 1.

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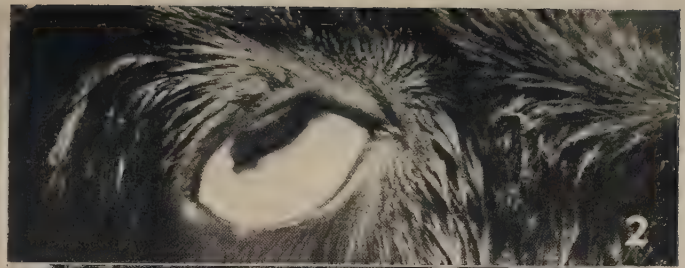

FIG. 1. "Eye reaction" in right eye of an experimentally infected puppy after the conjunctival instillation of the virus. Left eye normal.

FIG. 2. A close-up view of the "eye reaction" showing oedema of conjunctiva and *membrana nictitans*.

FIG. 3. (*a*) Acute tonsilitis and lymphadenitis in a puppy killed one week after conjunctival instillation of the virus.
(*b*) Normal tonsils and lymph nodes of a puppy killed 2–4 weeks after instillation of the virus.

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BILE PIGMENT METABOLISM IN CATTLE

Disturbances in Bile Pigment Metabolism in Disease

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INTRODUCTION

During the course of an investigation into the possible value of liver function tests in cattle (Garner, 1951), changes in serum bilirubin and in urinary bilirubin and urobilin levels were studied in a variety of pathological conditions. The opportunity was also taken to investigate the behaviour of the "direct" and "indirect" van den Bergh reactions in cattle serum.

METHODS

A description of the experimental animals used and of the method of classification of the hepatic lesions encountered has been given elsewhere (Garner, 1952a).

Icteric index. This was determined photometrically by the technique of Hawk, Oser and Summerson (1947) using the Hilger-Spekker photo-electric absorptiometer and Ilford 601 (Spectrum violet) filters.

Serum bilirubin. Bilirubin was estimated by two modifications of the van den Bergh reaction (a) that of Haslewood and King (1937) as modified for use with the photoelectric colorimeter (King, 1946). A blank determination was carried out on each serum sample using 0·5ml. of 0·25N hydrochloric acid in place of the diazo reagent. Readings were made in the Spekker instrument with lcm. cells and Ilford 605 (Spectrum yellow-green) filters against the usual methyl red standard (b) that of Malloy and Evelyn (1937), the volume of reagents and serum being increased to give a final volume of 25ml. to allow the use of 4 cm. cells in the Spekker absorptiometer. Bilirubin for the preparation of a calibration curve was obtained from ox bile. For the direct reaction, water was substituted for methanol.

Urinary bilirubin. Qualitative methods only were used and included Fouchet's (Harrison, 1947), Hunter's (1930) and Yudkin's (1945) tests.

Urinary urobilin. The semi-quantitative method of Wallace and Diamond (1925) was employed with Schlesinger's test (Naumann, 1936) as an adjunct.

RESULTS

Use of the Icteric Index in Cattle. Fig. 1 compares the Icteric Index of a small series of cattle sera with the bilirubin content obtained using the method of Malloy and Evelyn (above). It is evident that not only is there no correlation between the index and bilirubin concentration, but also that the Icteric Index, as applied to cattle, may be grossly misleading. The colour of the serum, in fact, parallels the carotene and not the bile pigment content (Wilson, 1949). This point is not emphasized in most veterinary text-books. Both Coffin (1945) and Hutyra, Marek and Manninger (1946) describe methods for the determination of the Icteric Index of serum, but neither mentions that carotenoids may invalidate the results.

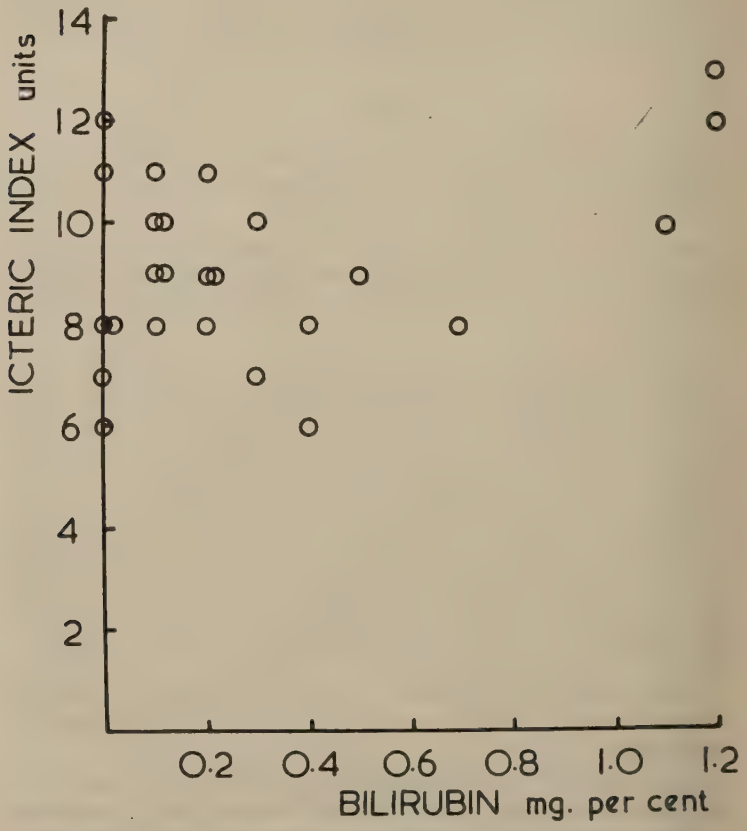

Fig. 1. Comparison of the Icteric Index and Bilirubin Content of Cattle Serum.

Bile Pigments in Cattle Serum. The bilirubin content of "normal" cattle serum is very small. As determined by the method of Haslewood and King, traces only are present. Ramsay (1946), using this method, was unable to demonstrate even the slightest trace of bilirubin. With the more sensitive method of Malloy and Evelyn, the mean value of 0·3mg. per cent for 102 sera has been found (Table I). As far as pathological conditions are concerned, an increase in serum bilirubin has been observed only as a result of haemolytic disease (particularly Anaplasmosis). The slight differences recorded in Table I are statistically insignificant.

TABLE I

BILIRUBIN CONTENT OF THE SERUM OF NORMAL CATTLE, OF CATTLE WITH LIVER DISEASE, AND OF CATTLE WITH HAEMOLYTIC JAUNDICE

Determined by the method of Malloy and Evelyn (1937). The figures in brackets were obtained using the method of Haslewood and King, (1937)

	Number of animals	*Serum Bilirubin (mg./100ml.)*	
		Range	*Mean ± S.D.*
Normal animals.. ..	102	0—1·4	0·31 ±0·168
	(146	0—0·6	0·02 ±0·062)
Diffuse hepatic lesions,			
Mild ..	17	0—2·4	0·53 ±0·031
	(32	0—3·5	0·13 ±0·607)
Severe ..	10	0—1·2	0·54 ±0·034
	(20	0—2·5	0·17 ±0·055)
Patchy hepatic lesions,			
Mild ..	3	0—2·7	1·13
	(3	0—2·5	0·83)
Severe ..	2	0·7,0·9	0·80
	(2	—	0·00)
Biliary obstruction ..	2	0·3,0·3	0·30
	(2	0·0,0·2	0·10)
Haemolytic jaundice ..	2	2·3,7·7	5·00

No biliverdin could be detected in 31 serum samples from normal English cattle using the method of Larsen, Evans and Watson (1947).

"Direct" and "Indirect" Bilirubin in Cattle Serum. The course of the direct and indirect van den Bergh reactions has been studied in the serum from two cattle with haemolytic jaundice and from four with clinically diagnosed liver disease. To facilitate comparison of the results with those obtained in human serum, the "direct-" and "indirect-quotients" used by Gray and Whidborne (1947) were calculated. Fig. 2 shows graphs of (D.Q.)t and (I.Q.)t plotted against time for the six animals mentioned above. These curves are almost identical with those obtained by Gray and Whidborne (1947) in human cases of haemolytic and hepatic disease.

Urinary Bile Pigments. Bilirubin was detected in one only of 55 specimens of cattle urine examined. This animal was heavily infected with *T. congolense* and had a serum bilirubin content of

2·4mg. per cent. The liver lesion was classified as "diffuse mild". In view of the otherwise low levels of serum bilirubin, these results were not unexpected.

"Urobilin" (a collective term used here to include stercobilinogen, stercobilin, mesobilirubinogen, and mesobilene) was found to be present inconstantly in the urine of both normal and diseased

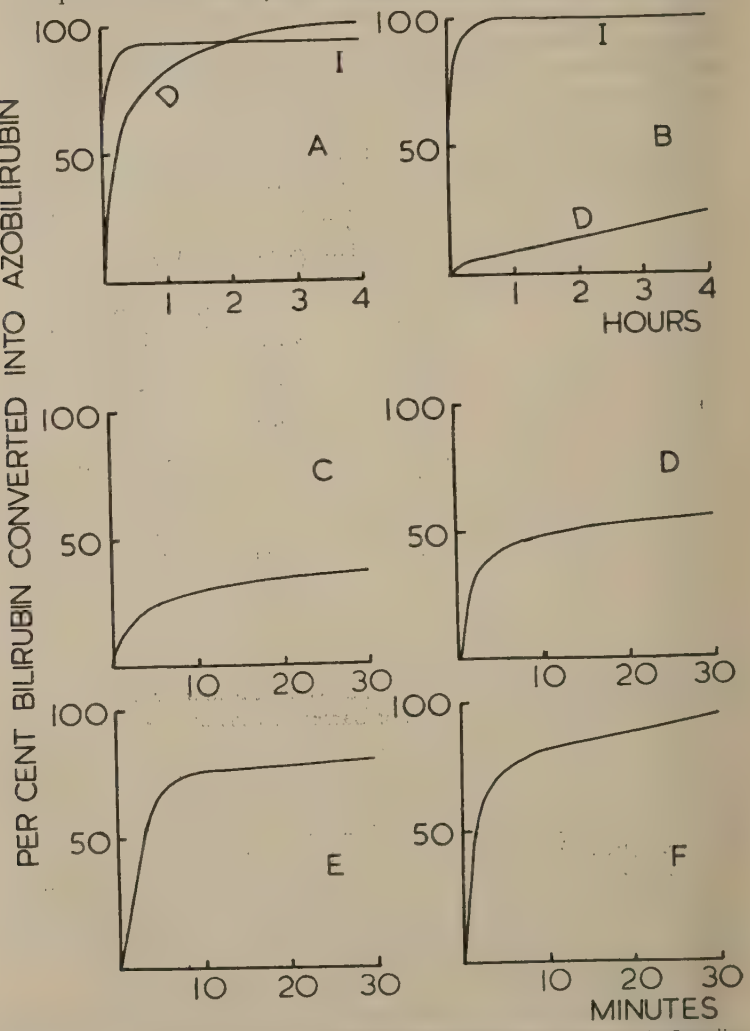

Fig. 2. $(D.Q.)_t$ and $(I.Q.)_t$ curves obtained from Cattle with Haemolytic Jaundice (A and B) and $(D.Q.)_t$ curves from Cattle with Liver Disease (C, D, E and F).

cattle. This result is in accordance with the findings of Kühle (1937), who reported that neither bilirubinuria nor urobilinuria was indicative of liver disease in cattle, since both pigments were found to occur sporadically in the urine of cattle with healthy and diseased livers.

DISCUSSION

Since bile pigment metabolism in cattle appears to differ to no major degree from that in man (Garner, 1952d.), the consequences of deviations from the normal would be expected to be much the same. But, as revealed by the results presented above, these consequences are of greatly reduced significance in the bovine. Severe generalized liver disease has been observed to produce only a slight increase in the serum bilirubin concentration, the maximum figure being 3·5mg. per cent (Table I). Similar values have been given by other workers: thus Plowright, Burdin and Thorold (1952) recorded in one case the "high figure" of 3·2mg. per cent. In man, values of 10mg. per cent or more are more commonly associated with liver disease of this kind.

Both the figures quoted were obtained using techniques which involved precipitation of the serum proteins. Such procedures bring about the loss of some of the bilirubin by absorption on to the protein precipitate. Gray and Whidborne (1946) reported losses of up to 50 per cent using the method of Haslewood and King (1937) as compared with that of Malloy and Evelyn (1937). With the low values encountered in normal cattle serum, even greater losses were obtained. Such losses may explain the failure of Ramsay (1946) to demonstrate, using the method of Haslewood and King, even the slightest trace of bilirubin in normal cattle serum, leading him to the conclusion that bilirubin played no part in the normal metabolism of cattle. Although losses of this kind may account to some slight extent for the relatively low figures found in pathological conditions in cattle, they cannot account for the quite considerable differences observed between analogous conditions in man and in the bovine.

It has been suggested (Green, 1951) that the bilirubin of cattle serum reacts in some way differently towards the van den Bergh reagent. There is, however, no evidence that this is indeed the case. The serum bilirubin in liver disease gives a direct reaction (Plowright *et al.*, 1952) and in haemolytic disease an indirect reaction, the course of the reaction in each case being very similar to that recorded in the human subject (Fig. 2). Further, bilirubin forms complexes with bovine serum proteins exactly similar to those formed with human serum proteins (Martin, 1949).

From this evidence it can be concluded that the bovine liver possesses a very large functional reserve, particularly with regard to its capacity to excrete bilirubin. Serum alkaline phosphatase (Garner, 1952b) and certain dyes (Garner, 1952c) appear to be dealt with with equal facility, although here there is some evidence that the excretary function may be slightly impaired when the hepatic lesions are sufficiently severe and widespread. Since urinary urobilin represents that part of the total urobilin reabsorbed from the intestine which has escaped removal from the portal system by the liver, the sporadic occurrence of urobilin in cattle urine suggests that this substance may sometimes be produced in quantities sufficient to exceed the capacity of the liver to excrete it. However, in the case of bilirubin, it appears that it is only when almost every liver cell is affected, as by dimidium bromide (Plowright *et al.*, 1952), in ragwort poisoning (Nicholson, 1945) or by carbon tetrachloride (Garner, 1952a), that any noticeable deviation from normalcy occurs and, even then, the changes produced are relatively slight.

SUMMARY

A small amount of bilirubin and no biliverdin is normally present in cattle serum.

A significant increase in serum bilirubin is observed only in cases of haemolytic disease.

The relatively insignificant changes observed in liver disease cannot be accounted for by a difference in behaviour of cattle serum bilirubin, as compared with human serum bilirubin, towards the van den Bergh reagent.

It is concluded that the bovine liver possesses a very great reserve capacity in so far as its excretory function is concerned. This is particularly true with respect to bilirubin.

REFERENCES

Coffin, D. L. (1945). *Manual of Veterinary Clinical Pathology*, 2nd ed. New York : Comstock.

Garner, R. J. (1951). *Fellowship thesis*, Royal College of Veterinary Surgeons; (1952a). *J. comp. Path.*, **62**, 279; (1952b). *Ibid.*, 287; (1952c). *Ibid.*, 306; (1952d). *M.V.Sc. thesis*, Liverpool University.

Gray, C. H., and Whidborne, J. (1946). *Biochem J.*, **40**, 81; (1947). *Ibid.*, **41**, 155.

Green, H. H. (1951). *Personal communication.*

Harrison, G. A. (1947). *Chemical Methods in Clinical Medicine*, 3rd ed. London : Churchill.

Haslewood, G. A. D., and King, E. J. (1937). *Biochem.* J., **31**, 920.

Hawk, P. B., Oser, B. L., and Summerson, W. N. (1947). *Practical Physiological Chemistry*, 12th ed. London : Churchill.

Hunter, G. (1930). *Canad. med. Ass. J.*, **23**, 823.

Hutyra, F., Marek, J., and Manninger, R. (1946). *Special Pathology and Therapeutics of the Diseases of Domestic Animals,* 5th English ed. London: Bailliere, Tindall, and Cox.
King, E. J. (1946). *Micro-analysis in Medical Biochemistry.* London : Churchill.
Kühle, E. (1937). *Inaug. Diss.,* Berlin (abs. in *Vet. Bull.,* 1938, **8**, 77).
Larsen, E. A., Evans, G. T., and Watson, C. J. (1947). *J. lab. clin. Med.,* **32**, 481.
Malloy, H. T., and Evelyn, K. A. (1937). *J. biol. Chem.,* **119**, 461.
Martin, N. H. (1949). *J. Amer. chem. Soc.,* **71**, 1230.
Naumann, H. N. (1936). *Biochem. J.,* **30**, 347.
Nicholson, J. A. (1945). *Lander's Veterinary Toxicology,* 3rd ed. London : Baillière, Tindall, and Cox.
Plowright, W., Burdin, M. L., and Thorold, P. W. (1952). *J. comp. Path.,* **62**, 141.
Ramsay, W. N. M. (1946). *Vet. J.,* **102**, 206.
Wallace, G. B., and Diamond, J. S. (1925). *Arch. int. Med.,* **35**, 698.
Wilson, A. A. (1949). *M.V.Sc. thesis,* Liverpool University.
Yudkin, J. (1945). *Brit. med. J.,* **1**, 445.

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